Protease Cathepsins in Cardiomyopathy: From Mechanism to Intervention

21

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Abstract

Cardiovascular disease is the leading cause of death in the United States. Risk factors that contribute to the heart disease and cardiac dysfunction include diabetes, obesity, hypertension, high blood cholesterol, smoking, alcohol abuse, sedentary life style, unhealthy diet, family history, and aging. Whereas obesity and uncontrolled hypertension can lead to hypertrophic cardiomyopathy; chronic alcohol consumption and diabetes can cause dilated cardiomyopathy, both of which can eventually result in an impaired cardiac function and heart failure. Cathepsins are lysosomal proteases that are capable of degrading proteins. Studies have shown that cathepsins, particularly those that belong to the cysteine protease family exhibit an important role in the development of cardiomyopathy and heart failure, probably by regulating cardiac remodeling. In diabetic cardiomyopathy, cathepsin K, the most potent cathepsin in terms of its collagenolytic and elastolytic properties, regulates calcineurin/NFAT transcriptional signaling critical for cardiac remodeling. Under obese conditions, inhibition of cathepsin K results in cardioprotection. Cathepsins also exhibit potential effects on epigenetics associated with alcoholic cardiomyopathy. Therefore, targeting cathepsins may represent a novel therapeutic strategy for the prevention and/or treatment of cardiovascular diseases.

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S. Chakraborti et al. (eds.), *Proteases in Human Diseases*,
DOI 10.1007/978-981-10-3162-5_21

Keywords
Cathepsins · Cardiomyopathy · Heart failure · Mechanism · Epigenetics · Therapeutic strategy

1 Introduction

Cardiovascular disease is the leading cause of morbidity and mortality in the United States [1]. Diabetes, obesity, and alcoholism are among the major risk factors that contribute to heart diseases in the modern society. According to the statistics from Centers for Disease Control and Prevention (CDC), in the year 2012, more than 29 million people or 9.3% of the U.S. population was diabetic. Diabetic subjects are at higher risk for heart attack, congestive heart failure, and atherosclerotic disease all of which can lead to diabetic cardiomyopathy and heart failure [2]. Alcohol abuse is another notable problem worldwide. Chronic alcohol consumption can result in alcoholic cardiomyopathy, characterized by thinned and enlarged heart (dilated cardiomyopathy), disruption of myofibrillary architecture, and cardiac contractile anomalies [3]. Current therapeutic options for dilated cardiomyopathy, such as diabetic cardiomyopathy and alcoholic cardiomyopathy, are limited. Moreover, obesity is an emerging health problem worldwide and is an independent risk factor for developing cardiovascular diseases. Obesity-associated myocardial damage is characterized by cardiac hypertrophy and contractile dysfunction, which is referred to as hypertrophic cardiomyopathy. The numerous preclinical and clinical studies aimed at preventing and/or treating cardiac disorders have not made any dent on the staggering numbers of cardiomyopathies, warranting newer pharmacological strategies to address this problem. This review aims at a brief discussion of the mechanisms leading to the progression of cardiomyopathy under diabetic, obesity, and alcoholic conditions, and addresses the possibility of employing cathepsins as novel targets for prevention and/or treatment of cardiomyopathy.

2 Biological Properties and Functions of Protease Cathepsins

Cathepsins are proteolytic enzymes that are involved in lysosomal protein degradation, which plays a vital role in physiological and pathological processes in living organisms [4, 5]. Dysregulation of cathepsins have been shown to correlate with numbers of diseases such as arthritis [6, 7], cancer [8, 9], autoimmune disease [10], stroke [11], neurodegenerative diseases [12, 13], gastrointestinal diseases [14–16], cardiovascular diseases [17], diabetes, and obesity [18, 19]. The proteolytic property of cathepsins rely on their broad specificities, thus the cleavage sites are different among different cathepsins. Most cathepsins are endopeptidases that catalyze the cleavage of nonterminal amino acids or break peptide bonds within the

target sequences. A few of them are carboxy- or amino-peptidases that cleave the peptide bond at only carboxy- or amino-terminal residues. A main physiological role of cathepsins is protein turnover in the lysosome [5]. Generally, cathepsins are contained and activated within the acidic pH of the lysosomes, sparing the cytosol and membrane of the cells from proteolysis. However, impaired lysosomal integrity leads to leakage of cathepsins to the cytosol and eventually outside of the cell resulting in degradation of cellular components or proteins in the extracellular matrix (ECM) [20].

Cathepsin B, C, F, H, K, L, O, S, V, W, X, and Z, are cysteine proteases with cysteine residue in their catalytic site. Histidine residue in the active site can also assist in the hydrolysis of target peptide bonds on the substrate, as evidenced in cathepsin B His197 or His199, and cathepsin H His166. Other cathepsins are serine protease (cathepsin G), aspartyl proteases (cathepsins D and E), and exopeptidase (cathepsin A). The knowledge of the catalytic sites of different cathepsins is of great importance in developing specific inhibitors for these proteases [5, 21]. Cathepsin K is by far the most potent mammalian cysteine protease [5]. It hydrolyzes various synthetic substrates such as Z-Gly-pro-Arg-MCA, Z-Arg-Phe-AMC, Z-Arg-Arg-AMC, and Bz-Val-Lys-Lys-Arg-AMC [22, 23]. In contrast to the cathepsins B, L, and S, cathepsin K is predominantly present in osteoclasts, and has strong elastase and collagenase properties for the degradation of bone collagen, indicating a special role in bone resorption. Therefore, cathepsin K has been implicated in the pathophysiology of osteoporosis and arthritis [24, 25].

3 Cathepsins in Cardiomyopathies and Heart Failure

Cardiomyopathy, literally "heart muscle disease," is a chronic and sometimes progressive disease of the myocardium (heart muscle) that is abnormally enlarged, thickened, and/or stiffened. The most common case of cardiomyopathy is dilated cardiomyopathy, and posteriorly hypertrophic cardiomyopathy. The weakened heart muscle in these conditions is unable to pump blood to the rest of the body. Cardiomyopathy is caused by a range of risk factors including heredity, coronary heart disease (e.g., atherosclerosis), amyloidosis, diabetes, obesity, long-term alcoholism, endocrine diseases, sarcoidosis, hypertension, and certain drugs (e.g., doxorubicin). All these can lead to peripheral edema, irregular heartbeat, a heart valve problem, heart failure, or other complications.

Cathepsins are ubiquitously expressed in various tissues and play important roles in cardiovascular diseases [17, 26]. Alterations of both extra and intracellular proteolytic activities are invariably observed in heart failure and have been linked to hypertrophic cardiomyopathy, dilated cardiomyopathy, hypertensive cardiomyopathy, ischemic cardiomyopathy, and diabetic cardiomyopathy [27]. Cathepsins B, L, and S are capable of regulating autophagy [28–30], ECM turnover, antigen presentation, neuropeptide and hormone processing, inflammatory response, and apoptosis [10, 26]. Previous studies have shown that the expression and activity of

cathepsin, B, D, K, and S were elevated in atherosclerotic plaque and in the hypertrophic and failing heart in both human and animal models [7, 54, 55]. Cathepsin S and G also displayed detrimental effects by altering ECM degradation, and causing cardiac remodeling [31, 32]. On the contrary, knockout of cathepsin L in mice resulted in dilated cardiomyopathy, whereas over expression of cathepsin L displayed decreased inflammation, fibrosis, and cardiac hypertrophy, probably through AKT/GSK3 beta pathway [33]. These studies strongly suggest a pivotal role for cathepsins in cardiac remodeling and heart failure and attribute protective and detrimental roles for these cysteine proteases. Despite the growing number of recent studies on the role of cathepsins in cardiovascular disease, the cellular and molecular mechanisms by which cathepsin K regulates cardiac dysfunction in the setting of cardiomyopathy and heart failure are yet to be explored. Growing evidence suggests that the expression and activity of cathepsin K are elevated in both clinical and experimental models of neointimal lesions, atherosclerosis, coronary artery disease, hypertrophy, and heart failure [34–37]. Our recent studies have suggested that cathepsin K protein levels were markedly upregulated in human hearts of end-stage dilated cardiomyopathy, and deletion of *ctsk* gene protected against cardiac anomalies induced by pressure overload or high-fat diet (HFD) feeding in mice [38, 39]. We also found that *ctsk* knockout exhibited an overall improvement in systemic glucose utilization [39], which was consistent with the evidence that cathepsin K displayed a negative effect on glucose and lipid metabolism, and inhibition of cathepsin K attenuated body weight gain, elevated serum glucose, and insulin levels in obese mice [19, 40, 41]. Cathepsin K may therefore represent a potential target for prevention or treatment of cardiac hypertrophy and heart failure.

4 Cathepsin K and Calcineurin/NFAT Signaling in Diabetic Cardiomyopathy

Myopathic state of the heart in diabetic subjects is manifested as left ventricular dilation, impaired left ventricular contractility, reduced ejection fraction and cardiac output, cardiac compensatory hypertrophy, and enhanced risk of stroke and hypertension, eventually leading to maladaptation and heart failure [42]. Micro/macrovascular complications also contribute to the cardiac anomalies associated with diabetes [43, 44]. However, the explicit mechanisms underlying the disease are still controversial as the pathogenesis of diabetic cardiomyopathy is multifactorial. Myocardial contractile dysfunction can be attributed to structural changes in the heart as a result of atherosclerosis and hypertension. Recent evidence suggests that diabetes affects cardiac structure and function in the absence of coronary artery disease, valvular disease, or high blood pressure [45]. The general triggering mechanisms behind the complicacy of diabetic cardiomyopathy include metabolic disturbances, altered cellular insulin signaling, small vessel diseases, and myocardial fibrosis which mainly associated with the stimulation of renin–angiotensin–aldosterone system (RAAS) and increased cytokines. Additionally, cardiac autonomic neuropathy,

autophagy, and epigenetics may also contribute to the pathogenesis of diabetic cardiomyopathy [46, 47]. Studies have shown that cathepsin D accelerates cardiac muscle degradation that occurs in the late stage of diabetic cardiomyopathy by triggering autophagy [48, 49]. Impairment of cathepsin L by hyperglycemia has been suggested as a cause of poor neovascularization and regeneration capacity of ischemic tissues in diabetics [50].

Hyperinsulinemia under diabetic conditions contributes to cardiac hypertrophy and remodeling, which can be explained, at least in part, to the inactivation of glycogen synthases kinase-3β (GSK-3β), a well-recognized antagonist of the calcineurin, which in turn inhibits nuclear transcription governing the hypertrophic process via the nuclear factor of activated T cells (NFAT) [51, 52]. It has been demonstrated that a transgene encoding a constitutively active form of calcineurin was sufficient to induce cardiac hypertrophy that progressed to dilated cardiomyopathy, heart failure, and sudden death in transgenic mice [53]. Suppression of calcineurin activity or NFAT transcription inhibits brain natriuretic peptide (BNP) induction and cardiac hypertrophy [52, 54], indicating a potential therapy strategy targeting on calcineurin/NFAT signaling.

Calcineurin is a Ca^{2+}/calmodulin-dependent serine/threonine-protein phosphatase ubiquitously expressed in eukaryotic cells, and involves in a number of cellular processes including Ca^{2+} dependent signaling pathways. In skeletal muscle, calcineurin can modulate fiber type-specific gene expression which is dependent on Ca^{2+} signaling and contractile activity [55, 56]. Indeed, calcineurin has been shown to influence Ca^{2+} fluxes by modulating the activities of L-type Ca^{2+} channel [57], ryanodine receptor (RyR)/Ca^{2+}-release channels [58, 59], sarco/endoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA 2a) [60] and the inositol 1,4,5-triphosphate receptor [61] in the heart. It has been known that Ca^{2+}-mediated signal transduction is essential for cardiac remodeling and hypertrophy process, and the disturbance of Ca^{2+} homeostasis leads to contractile dysfunction of the cardiomyocyte [62]. Such alterations of Ca^{2+} signals could play a role in the pathophysiology of heart failure. Furthermore, decreased activity of cardiac L-type Ca^{2+} channel induces hypertrophy and heart failure through activation of calcineurin/NFAT signaling in mice [63].

Activation of calcineurin can dephosphorylate the regulatory domains of NFATs within the cytoplasm, and the translocation of dephosphorylated NFATs regulates gene expression in the nucleus [51]. Studies by Fiedlerm [52] and Gao [64] suggest that L-type Ca^{2+}-channel current can also cause NFAT activation. There are four calcineurin-regulated NFAT transcriptional factors, NFATc1-c4, each of which is present in the myocardium [65]. To date, NFATc3, and NFATc4 (NFAT3) have been shown as two main downstream targets of calcineurin for the initiation of hypertrophic response [66]. NFATc4 can in turn stimulate the transcription of pro-hypertrophic genes MEF2 and GATA4, thereby promoting pathological hypertrophy [67]. In addition, NFATc1 has been shown critical for endocardial valve remodeling, coronary vessels, and fibrous matrix formation in the maturing heart, and serves as an essential effector of receptor activator of NFκB ligand (RANKL) signaling, which in turn regulates cathepsin K expression [68, 69]. Research also indicated that in the cardiomyocytes, NFATs may interact with NFκB/p65 and induce

the nuclear translocation of NFκB, and genetic deletion of calcineurin/NFATs displays compromised NFκB transcriptional activation, which predisposes pressure overload-induced cardiac hypertrophy. On the other hand, full transcriptional activation of NFATs requires intact NFκB signaling and p65 transcriptional activity [70]. NFATs and NFκB have also been associated with apoptosis [71, 72].

Both cathepsin K and calcineurin/NFATs signaling pathway have been implicated as critical regulators of cardiomyocyte hypertrophy. It can be postulated that these two previously deemed independent signaling pathways may actually crosstalk with each other, based on the following suppositions: First, increased cathepsins K activity can lead to dysregulated glucose metabolism and increase in glucotoxicity, which can trigger calcineurin/NFATs signaling. Evidence suggests that both hyperinsulinemia and hyperglycemia can trigger calcineurin-NFATs pathway [16, 25, 78, 79]. In our studies, mice rendered diabetic by streptozotocin injection exhibited elevated levels of cardiac cathepsin K, whereas similarly treated cathepsin K knockout mice exhibited attenuation in fasting blood glucose levels and reduced cardiac calcineurin A expression. In addition, diabetic mice exhibited ventricular dilation and cardiac dysfunction that was markedly alleviated by cathepsin K deletion. Since insulin levels were decreased in mice subjected to streptozotocin treatment, it is likely that hyperglycemia and glucotoxicity as a consequence of streptozotocin challenge is the possible trigger for dilated cardiomyopathy and cardiac dysfunction, via the upregulation of cathepsin K. Second, higher level of cathepsin K may induce cardiac anomalies by dysregulation of calcium homeostasis which triggers calcineurin activation. Our studies have demonstrated that cathepsin K knockout dramatically reversed diabetes-induced reduction in SERCA2 and phosphorylation levels of phospholamban at Ser16 and Thr17, as well as attenuated diabetes-induced elevation of intracellular calcium concentration. Taken together, this would suggest that cathepsin K is an upstream signal for regulating Ca^{2+} flux, which contributes to calcineurin stimulation. It is likely that cathepsin K, by virtue of its protease function, cleaves calcineurin to its active form resulting in the activation of NFATs, and subsequently triggers diabetes-induced cardiac anomalies and cardiomyopathy. This may be akin to calpain, a Ca^{2+}-dependent cysteine protease that has been shown to directly cleave calcineurin into its active form both in vitro and in vivo [20]. Future work still needs to be done and to explore the specific cleavage sites of cathepsin K and the structural basis for activation of calcineurin.

5 Role of Cathepsin K in Obesity Cardiomyopathy and Cardiac Dysfunction

Obesity is an independent risk factor for the pathogenesis of cardiovascular diseases such as arteriosclerosis, coronary heart disease, hypertension, cardiomyopathy, and heart failure [73, 74]. Obesity increases the risk for high blood pressure, metabolic syndrome, and abnormal energy metabolism such as glucose intolerance,

dyslipidemia, and insulin resistance, all of which contribute to cardiac anomalies. In addition to genetic predisposition, both clinical and experimental evidence suggests a pivotal role of obesity in cardiac hypertrophy and myocardial dysfunction. Accumulating human studies also confirm that obese people are prone to heart failure [74, 75].

A number of molecular mechanisms including the alteration in cardiac substrate utilization, inflammation, oxidative stress, mitochondrial injury, apoptosis, disrupt of extracellular matrix, fibrosis, endoplasmic reticulum stress, leptin resistance, endothelial dysfunction, lipotoxicity, and impaired Ca^{2+} homeostasis have been speculated as causes for obesity-induced cardiac dysfunction [76–80]. It is speculated that both calcium-dependent CaMKK and calcineurin might participate in obesity-associated cardiac hypertrophy and cardiomyopathy [51, 81, 82]. Similar to diabetic cardiomyopathy, calcineurin may also play an essential role in transducing hypertrophic signals in obese individuals, partially by activating NFAT transcription factors, the signaling of which may involve cathepsin K.

Cathepsin B, L, and K have been shown to be positively associated with lipotoxicity, and cathepsin K negatively regulates lipid metabolism. According to Chiara and co-workers, mRNA levels of *ctsk*, as well as Mitf and TFE3, two transcription factors involved in *ctsk* induction in osteoclasts, were dramatically higher in white adipose tissue (WAT) of obese mice, compared to their wild-type littermates. Interestingly, mRNAs were attenuated in mice undergoing weight loss. *Ctsk* gene expression has been positively correlated with body mass index [41]. In human studies both cathepsin K protein and *ctsk* mRNA expression were elevated in the WAT of overweight/obese patients, supporting the notion that *ctsk* is a novel and reliable marker of adiposity [83]. Studies from our lab showed that *ctsk* knockout significantly attenuated HFD-induced obesity and cardiac dysfunction evidenced as cardiac hypertrophy, cardiomyocyte contractile dysfunction, impaired intracellular Ca^{2+} handling, and apoptosis [39].

Obesity is characterized by defective fat storage, increase in intracellular lipid accumulation, and dyslipidemia. Xiao and co-workers found that *ctsk* is involved in the pathogenesis of obesity by promoting adipocyte differentiation in both human and in cultured cells. Expression and activity of cathepsin K gradually elevates concomitant with the differentiation of 3T3-L1 pre-adipocytes into mature adipocyte [83]. Similar to matrix metalloproteinase (MMP)-2, -3, and -9, cathepsin K, as a cysteine protease, has ability to degrade certain components of the ECM, which contribute to the ECM remodeling and adipocyte differentiation, likely via regulation of peroxisome proliferator-activated receptors (PPAR) and/or CCAAT/enhancer-binding proteins (CEBPB) [84, 85]. In addition, osteonectin that modulates cell adhesion, differentiation, and angiogenesis can be cleaved by cathepsin K in the WAT, resulting in enhanced matrix plasticity, and facilitating adipose remodeling and angiogenesis. Funicello and co-workers [40] found that the rate of lipolysis in adipocytes together with CPT-1 activity were increased in both young and HFD-fed $ctsk^{-/-}$ mice compared to wild-type mice, suggesting an increased release and/or utilization of free fatty acid (FFA) by down-regulating cathepsin K. Furthermore, plasma levels of leptin and triglyceride were significantly lower in adult $ctsk^{-/-}$ mice. The authors concluded that

the absence of *ctsk* is associated with increased energy expenditure, which might be due to increased activation of brown adipose tissue (BAT) for thermogenesis. Studies from Podgorski and co-workers indicated an involvement of cathepsin K in the regulation of adiponectin, an adipokine with anti-inflammatory and anti-angiogenic properties, which is dramatically decreased in obesity [86].

Platt and co-authors revealed that cathepsin K expression is regulated by shear stress in cultured mouse aortic endothelial cells (MAECs) and is elevated in endothelium in human atherosclerosis. Elastase and gelatinase activity was also increased in MAECs exposed to shear stress, which was attenuated by knocking down *ctsk* with siRNA, suggesting that cathepsin K is a shear-sensitive protease [87]. Their study also showed a positive correlation between the cathepsin K expression in endothelium and the integrity of the elastic lamina. These findings suggest that cathepsin K may function as an ECM protease and is involved in arterial wall remodeling and atherosclerosis. Indeed, cathepsin K levels have been positively correlated to plaque volume, and blood levels of cathepsin K have been suggested as independent predictor of coronary artery disease [36].

Sustained obesity can lead to type 2 diabetes and dampened insulin signaling has been observed in the heart from HFD-fed mice. Knockout of cathepsin K improved cardiac function and insulin signaling and reduced apoptosis in obese mice [39]. Yang and co-workers have shown that inhibition of cathepsin K reduced serum glucose and insulin levels by degrading fibronectin [19]. Collectively, these studies suggest that cathepsin K plays may play a role in regulating insulin signaling and preventing apoptosis of the heart of obese mice.

In addition to the above mechanisms, cathepsin K inhibition prevents cardiac hypertrophy by alleviating cardiac remodeling, both in vivo and in vitro studies. Indeed, the stimulation of the mTOR and Erk signaling pathways both of which were induced in the hypertrophic heart was blunted by *ctsk* deletion [38]. HFD can induce not only increased body weight, but also increased heart weight, left ventricular wall thickness, excessive epicardial fat and fatty infiltration of the myocardium, as well as increased total blood volume and cardiac output, all of which contribute to cardiac dysfunction, cardiomyopathy and heart failure. Under these conditions, inhibition of cathepsin K protects against the development of obesity-associated cardiomyopathy via mitigating cardiac remodeling. Furthermore, cathepsin K inhibition by virtue of its beneficial effects on the vasculature can also attenuate obesity-induced hypertension [38]. Furthermore, studies form our lab demonstrated that inhibition of cathepsin K suppresses oxidative stress in the mouse heart and in cultured H9c2 cells.

6 Cathepsins, Alcoholic Cardiomyopathy, and Epigenetics

Alcohol abuse is a serious medical and social problem. Excessive or chronic alcohol intake can lead to alcoholic cardiomyopathy, a disorder of the heart muscle characterized by compensatory cardiac hypertrophy, left ventricular dilation,

impaired left ventricular contractility, reduced ejection fraction and cardiac output accompanied with myocardial fibrosis, cardiomyocyte apoptosis, and mitochondrial impairment. Cardiac remodeling and compensatory cardiac hypertrophy can eventually result in maladaptation and heart failure [88, 89]. However, the explicit mechanisms underlying the disease are yet unclear. A number of mechanisms including direct toxicity of ethanol, indirect toxicity through its metabolites [acetaldehyde and fatty acid ethyl esters (FAEEs)], oxidative stress and impaired autophagy may be involved in alcoholic complications. Acetaldehyde, the primary intermediate in the metabolism of ethanol, is an essential candidate toxin in developing alcoholic cardiomyopathy through hypertrophic responses, interruption of myocardial protein synthesis (as a result of adduct formation) and impairment of mitochondrial integrity [90]. Meanwhile, free radicals produced during ethanol metabolism and FAEEs are also important triggers for alcoholic heart diseases [91]. Additionally, racial and gender differences, genetic variation in certain myocardial proteins, genetic polymorphism of alcohol metabolizing enzymes, epigenetics, and alterations in the levels of microRNA levels may also contribute to the development of alcoholic cardiomyopathy [92].

Epigenetics is a science studying heritable change in the genome, which affects gene expression without any change in the DNA sequence. Potential epigenetic mechanisms include DNA methylation, histone modification, and RNA-based mechanisms such as microRNAs (miRNAs) and long non-coding RNAs (ncRNAs), leading to either transcriptional suppression or activation of the genes independently of the DNA genome sequence [93, 94]. Ethanol can induce epigenetic alterations in different immune cell types including granulocytes, macrophages, and T-lymphocytes which promote inflammation [95]. Epigenetic is emerging as a hot research topic and is a potential target for primary prevention or treating cardiovascular diseases. Epigenetic factors such as methylation and acetylation of histones have been shown to be correlated with enhanced expression of small non-protein-coding ribonucleic acids [92]. Preliminary studies have found that DNA methylation, histone modifications, and RNA-based mechanisms may have an association with the development of cardiac hypertrophy and heart failure. A recent study showed a significant difference in plasma microRNA profile between patients with alcoholic cardiomyopathy and a healthy population [96]. Our previous studies have suggested that ethanol feeding increased the levels of Beclin1 and triggered the formation of autophagosomes in cardiomyocytes, which results in myocardial contractile dysfunction through autophagy. Meanwhile, the expression of miR-30a, a target of Beclin1 was reduced in cardiomyocytes [90]. However, no active epigenetic agents or drugs targeting histone methylation and/or acetylation have actually reached clinical trials for cardiovascular diseases [93].

Generally, epigenetic alterations can be reversed via therapeutic approaches including but not limited in DNA methyltransferase (DNMT) inhibitors, histone deacetylase (HDAC) inhibitors, histone acetyltransferase (HAT) inhibitors, miRNA therapeutics and commonly used medicines like statins [97]. For example, polyphenols and folic acid may be as decent candidates to reduce lipid and ROS levels by regulating DNA methylation and histone modification. Resveratrol, a

DNMT inhibitor, can modulate sirtuin 1, MAP38 kinase, NF-κB, AP-1, eNOS and inflammatory cytokines. Moreover, the HAT inhibitor curcumin is a polyphenol and actually modulates various epigenetic factors such as HDAC, HAT, DNMT, and miRNAs [98]. Curcumin as an antioxidant may influence both acetylation and deacetylation by regulating oxidative stress. Trichostatin A, as a HDAC inhibitor, can also play a pivotal role in the prevention of cardiac performance and alleviate myocardial remodeling through stimulating endogenous cardiac regeneration [93]. Besides, the role of miRNAs in drug exploration as genetic targets has also been investigated. MicroRNAs are a class of short non-coding RNAs that target specific mRNAs thereby inducing degradation or translational inhibition during various physiological or pathological processes. Evidence indicated that miRNAs are involved in the actions of ethanol and play a key role in regulating the progression of cardiomyopathy. Ethanol can cause some miRNAs upregulated and others downregulated simultaneously [94]. For instance, upregulation of miR-212 by alcohol can lead to activation of fetal gene program and heart failure. Therefore, miR-212 can be a potential therapeutic target to protect the heart from chronic alcoholism. According to Jing and colleagues, nine differentially expressed miRNAs including miR-506, miR-1285, miR-512-3P, miR-138, miR-485-5P, miR-4262, miR-548c-3P, miR-548a-5P, and miR-K12-1 may be involved in the development of alcoholic cardiomyopathy. Particularly, miR-138, may be considered as a novel biomarker for the early diagnosis and treatment of human alcoholic cardiomyopathy [96]. Additionally, miR-340 was also regarded as a novel therapeutic target for the heart failure progression by restricting cardiac remodeling [99].

Cathepsin L has been shown to cleave histone H3 that is generated in vivo during mouse embryonic stem cell differentiation. In addition, it was demonstrated that an endogenous osteogenic growth peptide (OGP) that is identical to the histone H4 is responsible for bone regeneration [100]. These findings indicate that the proteolysis of H3 tail may associate with mammalian differentiation and a proliferative effect. Our study found that ethanol treatment increased osteoprotegerin (OPG) level in H9c2 myoblast, which was restored by pharmacological inhibitor of cathepsin K. OPG could increase OGP levels and was recently considered as a hypertrophic marker in patients with cardiomyopathy and heart failure, therefore we postulate that histone H3 and/or H4 may be regulated by cathepsins thereby regulating cardiac remodeling in alcoholic cardiomyopathy. Bulynko and co-workers speculated that cathepsin L-linked phenotypes such as defective skin and bone cell differentiation and dilated cardiomyopathy may result from cathepsin L deficiency-induced epigenetic heterochromatin changes in histone H3(K9) and the histone H2A.Z [101]. It has been suggested that *ctsk*, as a potential cardiovascular target gene, may be regulated by miR-107 that is upregulated in experimental models of heart failure, and gene of cathepsin S (*ctss*) has consensus binding sites for repressed miRNAs [102, 103]. MiR-212 may interact with cathepsin G in connection with collagen deposition [104].

7 Conclusion

Determination of the specific signaling pathways in the development of heart failure is essential for the discovery of novel therapeutic strategies. Cathepsins, especially cysteine cathepsins, are closely associated with cardiac remodeling and contribute to cardiomyopathy and heart failure. Cathepsins may also serve as diagnostic tools or biomarkers for heart failure. Cathepsin inhibitors have already been investigated in clinical trials for a variety of disease conditions such as osteoporosis and rheumatoid arthritis. These agents should be evaluated for their efficacy in preventing and/or treating cardiovascular diseases particularly the progression of heart failure. Inhibition of cathepsins may also alter genetic and epigenetic changes. Further studies are necessary to understand the broader implications and role of cathepsins to successfully target these proteases to treat or control heart disease.

Acknowledgements The authors acknowledge the support from the National Center for Research Resources (P20RR016474) and the National Institute of General Medical Sciences (P20GM103432). The authors have no other conflict of interest to declare. RG received a graduate student research award from the College of Health Sciences, University of Wyoming.

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