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Role of various proteases in cardiac remodeling and progression of heart failure

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Abstract It is believed that cardiac remodeling due to geometric and structural changes is a major mechanism for the progression of heart failure in different pathologies including hypertension, hypertrophic cardiomyopathy, dilated cardiomyopathy, diabetic cardiomyopathy, and myocardial infarction. Increases in the activities of proteolytic enzymes such as matrix metalloproteinases, calpains, cathepsins, and caspases contribute to the process of cardiac remodeling. In addition to modifying the extracellular matrix, both matrix metalloproteinases and cathepsins have been shown to affect the activities of subcellular organelles in cardiomyocytes. The activation of calpains and caspases has been identified to induce subcellular remodeling in failing hearts. Proteolytic activities associated with different proteins including caspases, calpain, and the ubiquitin–proteasome system have been shown to be involved in cardiomyocyte apoptosis, which is an integral part of cardiac remodeling. This article discusses and compares how the activities of various proteases are involved in different cardiac abnormalities with respect to alterations in apoptotic pathways, cardiac remodeling, and cardiac dysfunction. An imbalance appears to occur between the activities of some proteases and their endogenous inhibitors in various types of hypertrophied and failing hearts, and this is likely to further accentuate subcellular remodeling and cardiac dysfunction. The importance of inhibiting the activities of both extracellular and intracellular proteases specific to distinct etiologies, in attenuating cardiac remodeling and apoptosis as well as biochemical changes of subcellular organelles, in heart failure has been emphasized. It is suggested that combination therapy to inhibit different proteases may prove useful for the treatment of heart failure.

Keywords Heart failure · Diabetic cardiomyopathy · Dilated cardiomyopathy · Matrix metalloproteinases · Calpains and cathepsins · Cardiac caspases and apoptosis

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Introduction

Heart failure is a growing epidemic affecting approximately 23 million people worldwide [1]. Cardiac complications including cardiac hypertrophy, genetic cardiomyopathy, diabetic cardiomyopathy, dilated cardiomyopathy (DCM), and myocardial infarction (MI) are all known to eventually result in heart failure [1, 2]. The development of heart failure is invariably associated with increased levels of circulating catecholamines as a consequence of prolonged stimulation of the sympathetic nervous system [3, 4]. In addition, there occurs an activation of the renin-angiotensin system in heart failure which elevates the plasma levels of angiotensin (Ang) II [3, 4]. These hormonal changes increase both the preload and afterload on the heart and thus contribute to the geometric and structural cardiac remodeling [4], which is considered to be the underlying cause of heart failure. It has been suggested that the activation of different proteases, which are present in both the extracellular and intracellular environment of cardiomyocytes, is intimately involved in the process of cardiac remodeling and the occurrence of heart failure due to different etiologies [4–7]. Several lines of evidence have suggested that activation of proteases occurs due to oxidative stress and/or

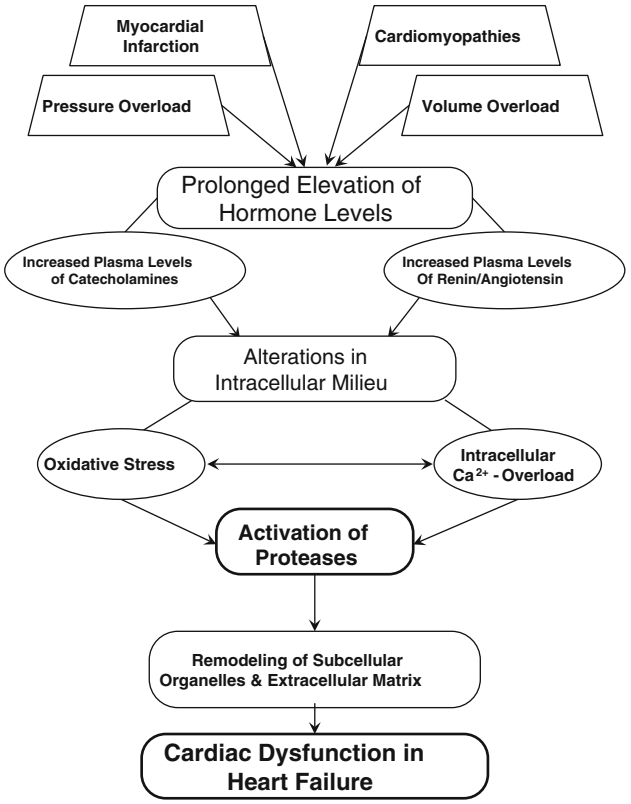

Fig. 1 Schematic representation for the involvement of activation of various proteases and cardiac dysfunction in different types of cardiovascular disease. Prolonged elevated levels of plasma hormones including catecholamines and renin-angiotensin are believed to produce oxidative stress and intracellular Ca^{2+}-overload, which may then serve as major mechanisms for the activation of different proteases

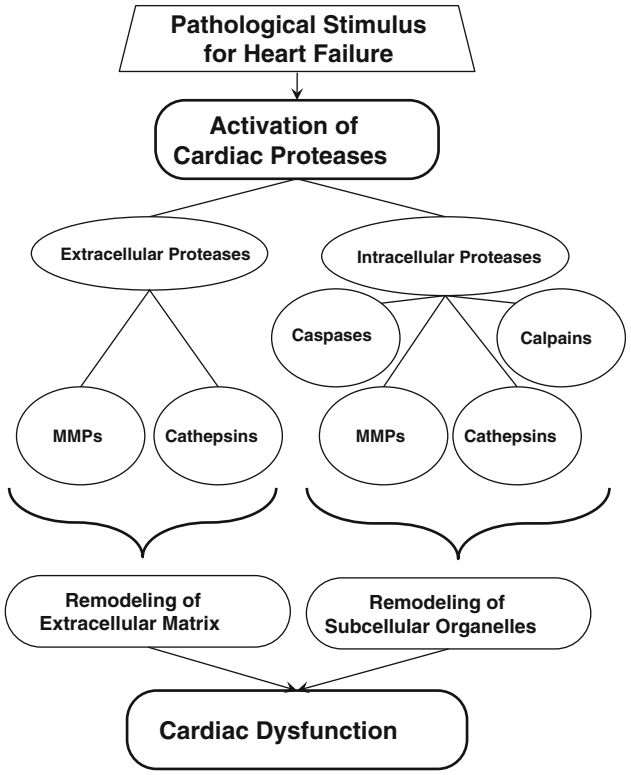

Fig. 2 Schematic representation of different families of proteases affecting the extracellular and intracellular environment in response to cardiac pathological stimuli. These various proteases result in remodeling of ECM and subcellular organelles leading to cardiac dysfunction. *MMPs* matrix metalloproteinases

intracellular Ca^{2+}-overload as a consequence of prolonged elevation of catecholamines, Ang II, and other vasoactive hormones in the circulation (Fig. 1) [3, 4]. Although the proteolytic activity allows the degradation of misfolded or malfunctioning proteins in cardiomyocytes and the extracellular matrix (ECM) under normal physiological conditions [7–9], different proteases, such as matrix metalloproteinases (MMPs), have become a topic of great interest regarding their involvement in biochemical remodeling of subcellular organelles and pathogenesis of heart disease [6, 7, 10–13]. In addition to MMPs, other protease families including calpains, cathepsins, and caspases have been considered to play an important role in the development of both geometric and biochemical remodeling as well as cardiac dysfunction in heart failure (Fig. 2). The activation and activities of calpains and cathepsins and their participation in cardiovascular disease have been extensively reviewed elsewhere [14–22]. It should be mentioned that the proteolytic activities of enzymes such as calpains and MMPs are controlled by endogenous inhibitors, including calpastatin and tissue inhibitors of metalloproteinases (TIMPs) [11, 12, 18, 19]; however, there are significant voids in the understanding of their role in the failing heart. In this article, it is planned to discuss the status of various extracellular and intracellular proteases in the failing heart due to hypertension and cardiac hypertrophy, different cardiomyopathies, and MI. Furthermore, the involvement of endogenous inhibitors in determining the extent of proteolytic activity in heart failure due to different etiologies will be described. Since apoptosis is associated with heart failure [3, 4], the participation of caspases and other proteases in the process of cardiac apoptosis will be highlighted. Although various proteases in the heart are activated under conditions of ischemia–reperfusion injury [5, 11, 12], this topic will not be dealt with in this review which is intended to focus on heart failure.

Proteases in hypertension and cardiac hypertrophy

Hypertension refers to increased arteriole pressure and total peripheral resistance as a result of vascular disease where hemodynamic overload on the heart is known to cause cardiac remodeling, cardiac hypertrophy, and heart failure [1, 23]. Changes in protease expression and activity have

been shown to occur in hypertension where protein levels of MMP-9 in the heart were increased, whereas those of TIMP-4 were reduced in Dahl salt-sensitive rats, a model of hypertension [24]. Decreases in protein serum concentrations of MMP-1 and increases in TIMP-1 were observed in patients with hypertensive heart disease [25–28]; however, one study has also shown that these levels remain unchanged [29]. Reduced turnover of collagen present in the interstitium [30] correlated with reduced MMP-1 and increased TIMP-1 protein levels in the serum of hypertensive patients both with and without cardiac hypertrophy [25]. In Dahl salt-sensitive rats, the mRNA levels and activities of MMP-2 and MMP-9 increased prior to the occurrence of left ventricular dilatation, systolic dysfunction, and pulmonary edema; cardiac remodeling was attenuated with the administration of ACE inhibitors indicating a correlation between hypertensive hormone stimuli and proteolytic remodeling [31]. In studies correlating hypertension with cardiac hypertrophy, the proneness of Dahl salt-sensitive rats to hypertrophy was attenuated by congenic transfer of TIMP-4, which restored cardiac function [24]. These observations in both hypertensive animal models and hypertensive patients indicate that various MMPs may be involved in the development of dilated and/or hypertrophic cardiomyopathy due to hypertension. It is pointed out that hypertension has also been shown to cause left ventricular hypertrophy and a reduction in calpain activity in both DOCA-salt hypertensive rat hearts and spontaneously hypertensive rat hearts [32]. On the other hand, both hypertensive patients as well as rats with heart failure showed elevated mRNA and protein levels of cathepsins S and K [33]. This is interesting because cathepsins are known to have significant ECM proteolytic capability by degrading elastin and fibrillar collagens and activating pro-MMPs [34]. Thus, it appears that increased activities of both cathepsins and MMPs, unlike the activity of calpain, may account for the high level of proteolytic activity for cardiac remodeling due to hypertensive stimuli.

In cardiac hypertrophy, a known precursor to heart failure, remodeling of the heart involves alterations in the ECM, including increased collagen deposition, as well as enlargement of the cardiomyocyte itself [23, 35, 36]. Although both extracellular and intracellular modifications by proteases are known to occur for obtaining the hypertrophic phenotype, mechanisms of these processes are not fully understood. Subcellular remodeling such as alterations of various isoforms of the Na^+-K^+-ATPase has been shown in both mild and severe hypertrophy with significant reductions in the protein content and mRNA level for the α_2 isoform of this enzyme [37]. Increased protein levels of TIMP-1 were evident upon attenuation of cardiac hypertrophy caused by MI using the K^+-ATP channel opener KMUP-3; decreased protein levels of MMP-9 and fibrosis were also observed [38]. Likewise, other studies indicated that TIMPs prevent the development of cardiac hypertrophy. When TIMP-4, the most abundant TIMP present in the heart, was knocked out in a pressure overload model, TIMP-2 compensated for the loss of TIMP-4 in maintaining cardiac histology, survival rate, and cardiac function compared with control [39]. It should be noted that when comparing eccentric versus concentric left ventricular hypertrophy, there were no differences in the serum levels of MMP-9 and TIMP-1, TIMP-2, and TIMP-4 suggesting that other proteases may be involved in determining these different phenotypes [40].

There was notable regression in hypertrophy as well as improved cardiac function in reverse remodeling upon heterotropic transplantation of hypertrophic hearts [41]. Changes such as decreased heart weight and cardiomyocyte area have been noted as possibly being a result of increased MMP-2 and MMP-9 expression during unloading, despite the continued increase in collagen deposition [42]. Increased protein levels of TIMP-1 were observed in both hypertrophied and unloaded hearts, suggesting the influence of changes in TIMPs in cardiac remodeling [42]. What is interesting to note is that cardiac hypertrophy in athletes showed comparable levels of TIMPs; however, both their MMP-2 and MMP-9 protein levels were lower in comparison to patients with pathological cardiac hypertrophy [43]. These data are consistent with another study evaluating patients with hypertrophic cardiomyopathy, which showed an increase in serum levels of both MMP-2 and MMP-9; however, only MMP-9 levels correlated with fibrosis [44]. It should be mentioned that cardiac hypertrophy is associated with increased MMP-2 and MMP-9 levels contributing to a significant imbalance between MMPs and their endogenous inhibitors, TIMPs. There is also the implication of an MMP profile change between hypertension and cardiac hypertrophy where the increased level of denatured collagen formed by MMP-1 prompts the increase in the levels of MMP-2 and MMP-9 to further degrade it without a concomitant increase in the level of TIMPs. Nonetheless, further studies are needed to clarify the relationship of MMPs and TIMPs in fibrosis and cardiac hypertrophy.

In addition to the alterations in the levels of MMPs and their endogenous inhibitors, there are other proteases that have been studied with respect to cardiac hypertrophy. In a feline right ventricular pressure overload model, calpain activity increased with an inverse relation to calpastatin level at 24–48 h; however, a week later, these changes returned to basal levels [45]. When increased levels of calpastatin were incorporated into the genome of mice, it was found that the decreased activation of calpain impaired NFκB activity and prevented Ang II-induced hypertrophy

[46]. Caspase-3 activation was also increased after inducing pressure overload, although when a caspase-inhibitor calpeptin was used, the activation of caspase-3 was attenuated but calpain activation was unaltered [45]. This indicates that calpain is a more critical protease in the development of cardiac hypertrophy, whereas the increase in caspase-3 activity may be more significant concerning the progression toward end-stage hypertrophy and the development of heart failure. The treatment with E64-c, a cysteine protease inhibitor, reduced the overall extent of cardiac hypertrophy due to isoproterenol as well as prevented the calpain-like activity [47]. These studies strengthen the view that calpain is involved in the initial development of cardiac hypertrophy in addition to changes in MMP proteolytic activity. Thus, it would be prudent to ascertain that the increases in both cardiomyocyte and overall heart size are associated with the increases in the activities of more than 1 family of proteases, and in fact, extensive work remains to be carried out to elucidate the pattern of temporal alterations in the activities of other proteases at different stages of this malignant phenomenon. In hypertension, it appears that remodeling occurs in part as a result of changes in MMP proteolytic activities, although there still remains to be investigated the cause and effects of cathepsin activity increase and calpain activity decrease. With regard to hypertrophy, the extent of remodeling occurs involving MMP-2 and MMP-9 as indicated in the study comparing athletic and pathological hypertrophy; however, proteases, such as caspase-3 and calpain, are emerging as potential players in the remodeling process as well.

Proteases in dilated cardiomyopathy

DCM is a heart-muscle disorder that can lead to heart failure where a portion of the myocardium is distended, with increases in interstitial fibrosis, wall thinning, chamber dilatation, and impaired contractile function [48]. The activities of different proteases have been shown to change depending upon the stage of the development of DCM. In general, MMP activities appear to contribute to the development of both MI-induced and idiopathic DCM as a result of their extensive ECM remodeling capability. MMP-1 and MMP-13 initially degrade both intact collagen and proteoglycans which are further altered by MMP-2/MMP-9 [12]. In a rat model of DCM induced by an injection of cardiac C protein [49], inflammation peaked at 2 weeks post-injection with a corresponding increase in the levels of MMP-2, MMP-9, and TIMP-1 mRNA [50]. During a shift to the fibrotic phase after 6 weeks, only the level of MMP-2 mRNA remained elevated. This study implies that in DCM, MMP-9 fosters disease progression during the early inflammatory phase, whereas MMP-2 remains involved for the entire development of DCM [50]. Another study found that MMP-9 protein content was increased, whereas the increase in MMP-2 activity was normalized at the end of an 8-week period post-aortic banding [51]. Although there are disparities in changes in MMP activities between these two experimental models, it is clear that differential activation of specific MMPs results in the progression of cardiac remodeling. In addition, increased levels of MMP-2, MMP-3, and MMP-9 have been observed in patients with DCM [52–54]. Interestingly, neither the levels of MMP-2 nor the levels of MMP-9 in DCM patients were indicated as being sufficient in predicting cardiac events, but the elevation of MMP-3 was demonstrated as being useful in predicting poor prognoses [55, 56]. In a genetic population study investigating idiopathic DCM, the promoter region of MMP-3 had increased frequency of a −1,171 5A allele which indicates that remodeling occurring in DCM could be MMP-3 specific. This study suggested that, due to the ability of MMP-3 to degrade a wide variety of ECM substrates including proteoglycans, fibronectin, and laminin as well as activating pro-MMPs, it could play a key role in both ECM remodeling and the activation of other MMPs involved in this process [57, 58]. It appears that increased MMP-3 activity may be an important parameter in determining whether or not a patient will acquire DCM and thus may serve as a specific biomarker for differentiating it from other types of cardiomyopathies. Although the increases in mRNA levels of TIMPs have been observed in the hearts of DCM patients [55], these may be increased at the transcriptional level because the protein content of TIMP-1 and TIMP-3 was decreased in advanced human DCM [54]. Furthermore, increased myocardial MT1–MMP protein levels have been noted in left ventricular biopsy tissue, which is intriguing when taking into consideration that the levels of MT1–MMP mRNA bound to active ribosomes were actually reduced [59]. This elevation of MT1–MMP could be explained by its potential to be internalized from the cell surface as its abundance was increased in the cytosolic component of myocardial fibroblasts in DCM patients [59, 60].

Interestingly, not all proteases are detrimental in DCM as indicated in cathepsin L-deficient mice where interstitial fibrosis, accumulation of undigested material in enlarged lysosomes, chamber dilatation, and impaired cardiac contraction were observed [61]. When cardiomyocyte-specific expression of murine cathepsin L was induced in these cathepsin L-deficient mice, changes in cardiac ultrastructure and function were recovered, but the fibrosis still persisted [62]. It is pointed out that when cathepsin L was overexpressed, not only are these pathologies reduced but there also was a significant attenuation in the activities of caspase-3, caspase-8, and caspase-9 [63]. This experimental model

illustrates the necessity for cathepsin L for the maintenance of lysosomes in order to prevent alterations in cardiac structure and function that can lead to DCM [61]. On the other hand, increased mRNA and protein levels of cathepsin L have been observed in DCM patients where the expression levels of cathepsin L mRNA correlated negatively with ejection fraction [64]. Cathepsin B mRNA and proteins levels have also shown to be inversely related with ejection fraction in patients [65]. Although these data comparing murine and human DCM appear contradictory, one must keep in mind that the activities of other proteases may be altered and the increased activity of cathepsins could be a result of the remodeling process. In addition, increases in cathepsin activity appear to be detrimental but the basal activity of cathepsin L may be protective against the progression toward DCM in humans. However, more research needs to be performed to determine the true role of cathepsins in the remodeling of ECM and subcellular organelles during the development of DCM. In DCM, a consistent distinguishing factor appears to be an increased activity of MMP-3 which has been shown to serve as a biomarker in patients. In addition, the importance of cathepsins, specifically cathepsin L, in maintaining lysosomal function is highlighted in DCM, so although increased activity of cathepsins should be avoided, complete attenuation of their activity could significantly impair cardiac function.

Proteases in diabetic cardiomyopathy

Insulin deficiency and/or insulin insensitivity in diabetes mellitus is known to cause an increase in circulating glucose, catecholamine levels, activation of the renin-angiotensin system, and a metabolic shift, which induce cardiovascular dysfunction called diabetic cardiomyopathy [66]. As a result of changes in the extracellular and intracellular environments, cardiac remodeling occurs in association with shortened left ventricular ejection time, longer pre-ejection period, and elevated end-diastolic pressure in diabetic mellitus patients [67]. Myocardial hypertrophy and significant myocytolytic changes are also observed in diabetic patients with congestive heart failure [68, 69]. In addition to cardiac remodeling, varying degrees of subcellular alterations have been shown to occur during diabetic cardiomyopathy, emphasizing oxidative stress as a mechanism of subcellular remodeling and heart dysfunction [66]. Changes in protease content and activities were also evident in the diabetic heart and are believed to participate in the ECM and subcellular remodeling processes [70–72]. There were decreased levels of MMP-2 activity and mRNA expression and increased levels of TIMP-2 mRNA expression in the myocardium of diabetic rats [70]; these alterations are considered to contribute to cardiac fibrosis [71, 72]. The decrease in MMP-2 could be due to deregulated ECM degradation as a result of decreased MT1–MMP protein expression [73]. It should be noted that MT1–MMP contributes to ECM degradation by creating a complex with TIMP-2 to activate MMP-2. Although TIMP-2 expression is increased in diabetic cardiomyopathy, the activity of MMP-2 is reduced because of the decrease in MT1–MMP expression [74]. Such a change appears to be specific to diabetic cardiomyopathy as it differs from what has been observed in both DCM and cardiac remodeling post-MI [73]. A reduction in MMP-2 activity correlating with decreased collagen turnover was evident in the Otsaku Long-Evans Tokushima fatty (OLETF) rat model, which is reminiscent of human Type 2 diabetes due to their spontaneous development [75–77]. The activity of MMP-7, a key regulator of fibrosis in the heart, and the activities of other MMPs were increased in the diabetic myocardium [78–80]. The accumulation of denatured collagen in fibrosis occurred partially as a result of increased activity of MMP-7, which cleaves ECM proteins resulting in denatured collagen, in diabetic patients with diastolic dysfunction, although an increase in the activity of MMP-9 was also found in these patients [81]. It is possible that the increase in MMP-9 activity is insufficient to cleave the accumulating collagen and preventing fibrosis in diabetic cardiomyopathy. Thus, the importance of differentiating MMP activities between diabetic cardiomyopathy and other cardiomyopathies is critical in targeting specific changes in order to potentially minimize the remodeling process in diabetic cardiomyopathy.

In addition to MMPs, changes in calpains, caspases, and cathepsins may also be involved in diabetic cardiomyopathy. Calpain-1 was found to decrease the Na^{+}-K^{+}-ATPase activity contributing to apoptosis in cardiomyocytes stimulated by high glucose concentrations [82]. In addition, it was observed that L-type Ca^{2+} channels and ryanodine receptors, when altered by reactive oxygen species, were involved in the activation of calpain-1; over-expression of calpastatin attenuated these effects [82]. There has also been observed increased caspase activation, specifically caspase-9, in diabetic cardiomyopathy [83–86]. There was a decrease in cathepsin D protein levels in young diabetic mice which rebounded by 24 weeks; this increase in lysosomal cathepsin D has been suggested to accelerate cardiac muscle degradation, which is common in the late stage of diabetic cardiomyopathy [87]. When evaluating how neovascularization is impaired in diabetes, it was found that the protein expression and activity of cathepsin L were reduced, significantly impairing ECM degradation and preventing endothelial progenitor cell-mediated neovascularization to occur, thereby contributing to the increased risk of ischemic heart disease [20, 88]. These studies indicate that the activities of different cathepsins

are altered to increase both the damage done directly unto the myocardium as well as the risk for further cell damage. Decreasing cathepsin D may protect the diabetic heart from significant injury [66]. As a result of the increased level of oxidative stress endured by the diabetic myocardium, the activation and action of proteases differs slightly from other cardiomyopathies; however, a significant amount of information remains to be acquired, especially pertaining to alterations in calpain activities and their possible role in subcellular remodeling and cardiac dysfunction.

Proteases in myocardial infarction and heart failure

Heart failure is the eventual endpoint of a number of cardiac pathologies where significant remodeling of both the ECM and subcellular organelles in the heart occurs in part by the activation of proteases and increased proteolytic activities. Proteases play an integral part in the remodeling process and have shown to be active by as early as 3 h post-MI concomitantly with decreased collagen integrity [89]. As a result of the decreased stability of the infarct zone due to these remodeling processes, heart failure as a result of rupture can occur [90]. These alterations are accentuated by the infiltration of inflammatory cells which further degrade both the ECM and intracellular proteins resulting in injury that involves changes in the infarct zone as well as throughout the viable ventricular myocardium [12]. Intracellularly, MI increases the level of β-myosin heavy chain (MHC) protein and decreases the level of α-MHC protein content [91]. This shift in the pattern of MHC proteins has also been observed in the hypertrophied heart due to MI at the mRNA level [92–94]. In addition to MHC, titin, a protein that provides elasticity to the sarcomeric contractile unit of the cardiomyocyte, is degraded in the failing heart resulting in impaired sarcomere contractility [95, 96]. Changes in Na^+-K^+-ATPase have also been noted as early as 6 weeks post-MI, with reductions in the α_2 isoform mRNA and protein levels, which mirrors what is occurring in both mild and severe hypertrophy [97]. Depression in Na^+-K^+-ATPase activity was also observed at 8 and 16 weeks post-MI [98], and it has been suggested that depressed Na^+-K^+-ATPase activity in cardiac hypertrophy due to MI could be a significant factor leading to heart failure. Levels of both SERCA mRNA and protein, as well as Na^+-Ca^{2+}-exchanger, were found to be decreased in the transition stage of MI-induced heart failure [99–105]. This suggests a flurry of proteolytic activity may be occurring inside the myocardium at the initial stages of heart failure which may provide a keystone target for attenuating the development of cardiac dysfunction.

The predominant amount of ECM remodeling in the progression to heart failure occurs as a result of increased MMP proteolytic activity and/or decreased levels of TIMPs [12]. In mice, a decrease in protein levels of protective TIMP-2 and TIMP-4 was noted in decompensatory heart failure along with an increase in MMP-2 protein content [51]. TIMP-4 has been shown to be significant in protecting the heart post-MI as demonstrated by a TIMP-4 knock-out model where MI increased the incidence of mortality predominantly as a result of left ventricular rupture [39]. In heart failure induced by pacing in a porcine model, increases in MMP-1, MMP-2, and MMP-3 protein content were 319, 194, and 493%, respectively, whereas gelatin and collagen III degradations were increased by 119 and 153%, respectively [106]. Interestingly, it was observed that post-MI, collagen accumulation was prevented and left ventricle enlargement was attenuated in an MMP-9 knock-out mouse [107]. Also, there was an increase in MMP-13 protein content and MMP-2 and MMP-9 proteolytic activities post-MI, with no significant change in mRNA. It was suggested that the remodeling environment may contribute to modification of these MMPs causing increased activation which was not attenuated by TIMPs, because TIMP-1 mRNA levels were initially elevated but slowly decreased at 5 weeks post-MI [108]. Increased promoter activity of MT1–MMP occurred in both infarct and remote myocardial regions at a rate of 20× at 3 days and 50× at 14 days post-MI [109]. This myocardial overexpression of MT–MMP has been shown to accentuate remodeling of the interstitium by triggering a pro-fibrotic response [109]. With regard to extracellular alterations, MMPs were of particular interest because several clinical trials measured MMP serum levels in heart failure patients. Increased left-sided filling pressures and collagen synthesis predominated due to excess TIMP-1 protein levels, despite increases in MMP-1, suggesting impaired collagen turnover [43]. Elevated levels of MMP-9 have also been linked to decreased left ventricular function and, specifically a concentration of 89.9 ng/ml in serum, indicated a decreased likelihood of survival in patients [110, 111]. In a recent study evaluating MMP levels in congestive heart failure patients, increased amounts of MMP-2, MMP-3, and MMP-9 were noted in the plasma, whereas plasma TIMP-1 was significantly less; lower fibronectin levels were also observed in these patients [112]. It is important to note that an imbalance between MMP activity and the rate of collagen deposition could lead to myocardial rupture, whereas necrosis present in the myocardium has been linked to increased chances of rupturing due to higher levels of MMP activity [113, 114]. Unfortunately, the majority of information available in the literature focuses on the proteolytic activities of a small subset of MMPs, particularly the gelatinases MMP-2 and MMP-9; however, these proteases are primarily responsible for the degradation of denatured collagen in the interstitium and are not

necessarily the MMPs responsible for causing its accumulation. Future studies must be performed in order to investigate how accumulation of denatured collagen occurs and elucidate whether other members of the MMP family are involved.

Other proteases also contribute in cardiac remodeling post-MI and heart failure. Increased level of calpain has been observed in patients with congestive heart failure with Ang II-induced cardiac remodeling [115]. Upon treatment of MI in a rat model with a combination of caspase-3 and calpain inhibitors, both the treated and control groups showed changes in the systolic blood pressure and heart rate, although these alterations returned to baseline [116]. This indicates that the initial changes occurring post-MI are not due to calpain or caspase-3 but their roles may be more pertinent to the progression of heart failure as their inhibition attenuated cardiac dysfunction. The level of the endogenous inhibitor of calpain, calpastatin, was unchanged in MI; however, the protein level of calpain was increased [117]. This suggests a common theme pertaining to the progression of heart failure indicating that an imbalance between proteases and their endogenous inhibitors is significant. In addition, both chymase and cathepsin G have been shown to increase Ang II formation in the failing human heart [118, 119], which further exacerbates the disease by increasing the level of oxidative stress and Ca^{2+}-overload. These alternative Ang-forming pathways provide insight as to why ACE-inhibitor therapy is not entirely preventative in reducing Ang II levels and subsequent remodeling leading to heart failure. In addition, the level of serine protease, proteinase 3, in the plasma of patients has been demonstrated as being important in determining the mortality and incidence of heart failure post-MI [120]. The alterations in proteolytic activity highlight how proteases are activated at varying times during cardiac remodeling, as evident by a study evaluating the inhibition of calpain and caspase-3. Preventing the formation of Ang II by inhibiting chymase and cathepsin G could prove as additional therapy in reducing the progression of heart failure. It should be noted that the development of heart failure is a complex process that involves numerous proteases; however, there is still much more research needed to be done in order to discover how and when these protease activities are altered and if there are other proteolytic enzymes contributing to cardiac dysfunction at this final stage. As the heart progresses toward heart failure due to MI, there is a significant increase in proteolytic activity, especially by the MMP family. In addition, an imbalance between the increased proteolytic activity of calpain and the unchanging level of its endogenous inhibitor, calpastatin, also contributes to cardiac dysfunction in the progression toward heart failure. Proteases, such as chymase and cathepsin G, are also responsible for the formation of Ang II so their inhibition could prevent the increase in Ang II and reduce its effects.

Proteases in apoptosis and cardiac remodeling

A common element in the progression of heart failure due to different cardiovascular etiologies, including cardiac hypertrophy and cardiomyopathies, is apoptosis. Apoptosis is a cell death mechanism primarily regulated by caspases, a family of 14 cysteine proteases that cleave their substrates specifically at an Asp residue [121]. The caspases are initially expressed as zymogens in the cytosol of the cell that become active after proteolytic cleavage at their Asp residues by a variety other proteases [121]. Both extrinsic (death receptor-mediated) and intrinsic (mitochondrial-mediated) apoptotic pathways activate caspases which subsequently degrade numerous polypeptides in the cell including major structural elements, DNA repair machinery, and protein kinases [121]. Another protease system, which may also be involved in the progression of apoptosis, is the ubiquitin–proteasome system (UPS). The proteasome is activated by a 700 kDa ATP-dependent complex known as the 19S regulatory particle with a subunit composition that varies with the physiological state of the cell and attaches to either one or both ends of the 20S proteasome regulatory unit [122–124]. In addition, the quantity of proteasome components is regulated by the gene expression, which has been observed to be upregulated during proteotoxic stress [124] that occurs during oxidative stress induced by cardiac pathologies including diabetic cardiomyopathy and MI [3, 66]. It is important to note that apoptosis has also been shown to occur independently of the caspase-mediated pathway via the apoptosis-inducing factor (AIF) [125]. In heart failure after MI in monkeys and patients, it has been observed that apoptosis in non-cardiomyocytes was nearly 9× greater than in cardiomyocytes, with most of apoptosis occurring in macrophages (41%) followed by neutrophils (18%), fibroblasts (16%), and then the remaining other cell types (25%) [126]. This opens a new avenue for studying the differences in apoptosis, primarily mediated by proteolytic activity, between various cell types and how oxidative stress causes differential apoptosis to occur within the medley of cells present in the heart. Nonetheless, it is becoming apparent that proteases other than caspases may also be involved in the development of cardiac apoptosis.

The extent of damage caused by cardiac injury is a factor of the extent of caspase-mediated apoptosis that occurs in different cell types within the heart. Furthermore, it is interesting that apoptosis occurs to a larger extent once blood flow is restored post-MI [127–130]; the extent of cardiomyocyte apoptosis is proportional to the extent of

cardiac injury. In MI patients, apoptosis appeared on the border lines of the infarct zone compared with the minimal amount in remote areas of the heart [131–134]. However, apoptotic diagnoses performed by using the TUNEL method, which employs immunohistochemistry for cleaved caspase-3, showed a lower apoptotic rate in human MI than previously reported [135]. It should be mentioned that caspases contribute to intracellular damage by cleaving contractile proteins including actin, myosin, and troponin in addition to pro-apoptotic factors that cause the release of cytochrome *c* from the mitochondria [136, 137]. In cardiomyocytes, the predominant apoptotic pathway appears to be intrinsic [138, 139], primarily through intermembrane space protein release, Bcl-2 protein involvement, and procaspase activation [140–142]. Caspase-3 has also been found to be associated with decreased left ventricular function due to its destructive nature of destabilizing sarcomeric structure [143] which is prevented by caspase inhibition that additionally attenuates ventricular remodeling [144–146]. When caspase-8 is inhibited by Z-IEDT.fmk, the effects of both caspase-8 and caspase-9 are affected, resulting in overall reduced BID cleavage and decreased cardiomyocyte apoptosis [147]. In patients with MI, there has been positive immunohistochemical staining observed for caspase-8 and caspase-9 in the border zone of MI; however, no significant change was observed when comparing patients with or without reperfusion treatment [121]. Positive staining of caspase-9 lining fibrotic scars post-MI in the human heart implies continuous apoptosis may contribute to ventricular remodeling after MI [121]. The reduction in cardiomyocyte apoptosis, size of MI, and improved heart function have been observed following inhibition with broad-spectrum caspase inhibitors, whereas selective caspase inhibitors depressed apoptosis without affecting the infarct size [148–152]. In addition to their pro-apoptotic activity, caspases have also been shown to activate MMPs, and their inhibition attenuated both regional and global LV remodeling in a porcine MI model [153]. When studying the transition from compensated hypertrophy to decompensated heart failure in a guinea pig model, there was a significant increase in the expression of the p17 subunit of caspase-3 [154]. In patients with DCM, both caspase-3 and caspase-9 were active [155]. In hypertrophied hearts due to AV shunt, the levels of both caspase-3 and caspase-9 were elevated in male rats with a corresponding increase in apoptosis; however, females had reduced cardiomyocyte apoptosis and did not exhibit heart failure [156, 157] although not all cardiomyopathies undergo caspase-regulated apoptosis. For example, hypertrophied cardiomyocytes from Dahl salt-sensitive rats were found to have a higher proportion of apoptosis initiated by AIF as opposed to caspase-mediated apoptosis [125]; inhibition of caspases by zVAD.fmk had no effect on AIF-induced apoptosis in these cardiomyocytes. On the other hand, inhibition of caspase activity may be a key in attenuating the extent of cardiomyocyte apoptosis during the progression of cardiac hypertrophy toward heart failure; thus, it is important to focus on preventing the extent of remodeling under situations showing increased caspase activity [121].

In addition to protein degradation via caspases, further downstream in the apoptotic pathway resides the destructive power of the UPS that is a primary non-lysosomal protein degradation pathway and is composed of the 26S-proteasome and ubiquitin [158]. The role of ubiquitin in the UPS is to act as a tag for the proteasome to identify how the protein targeted for proteolytic degradation is destroyed. The proteasome itself has three proteolytic activities ("trypsin-like," "chymotrypsin-like," and "caspase-like") that are assigned to one or more of the 7 β-type subunits [158]. The proteasome is made up of subunits that include the core 20S subunit and a regulatory 19S subunit made up of an additional 18 subunits arranged in a "base" and "lid" conformation [158]. The UPS system is responsible for regulating apoptosis by degrading caspases [159]. There is also an additional 11S activated proteasome that consists of a 20S proteasome docked with 1 or 2 11S activator rings or one 19S regulatory ring on one end and an 11S activator ring on the other [158, 160, 161]. Its 20S proteasome subunit specifically recognizes oxidized proteins that are of interest regarding cardiac pathologies where the heart endures oxidative damage as the 11S proteasome appears to focus on the degradation of damaged or senescent proteins and may be upregulated during periods of oxidative stress [162–164]. The proteolytic nature of the UPS system changes the molecular composition of the cell and has been indicated to alter its protein balances as a result of MI. Specifically, this has been demonstrated with the altered ratio of PKCε and PKCδ, which is important as PKCε appears cardioprotective and PKCδ may have both pro-apoptotic and pro-necrotic effects [165]. In addition, ischemic preconditioning prevented the decline of 26S ATP-dependent proteasome activity, thereby decreasing an accumulation of misfolded proteins as well as reducing the degradation of PKCε [165]. An accumulation of oxidized and ubiquitinated proteins appears to parallel with decreased 20 and 26S activities due to oxidative stress inactivation [166–168]. A possible hypothesis for the decreased core subunit activity could be a result of lack of ATP in ischemic cardiomyocytes which impairs the ATP-dependent activity of the UPS [167]. In addition, the proteasome subunit expression and activities are increased in cardiac hypertrophy [169, 170]. In the transition of hypertrophy to heart failure in patients, there was an increased level of ubiquitination during compensated hypertrophy followed by a 12× increase upon the onset of heart failure

[171]. The level of poly-ubiquitinated proteins was increased, and the enhancement of proteasome chymotrypsin-like activity was enhanced in DCM [155].

Inhibition of the proteasome has been shown to impair the heart function, as indicated by the use of bortezomib, an FDA-approved chemotherapeutic anti-cancer medication, which increases the occurrences of arrhythmias and heart failure [167, 172, 173]. However, other studies in rat, murine, and porcine models indicate that proteasome inhibition could be cardioprotective [174–179]. The pretreatment of isolated rat hearts with semi-selective proteasome inhibitor, lactacystin, increased the number of oxidized proteins; however, it was not found to significantly affect post-ischemic function [167, 180]. Sustained proteasome inhibition has been shown to attenuate cardiac hypertrophy, specifically by inhibiting the down-regulation of pro-hypertrophic signaling pathways including Akt, ERK1/2, calcineurin, and cyclones [181, 182]. Blocking proteasome degradation using antibacterial peptide, PR39, was observed to reduce ECM deposition by preventing downstream activation of NFκB, a factor involved in increasing cardiac fibrosis [183]. Proteasome inhibition has also been found to attenuate left ventricular remodeling in a pressure overload model by preventing further progression of hypertrophy, lessening the degree of collagen accumulation, decreasing cardiomyocyte apoptosis, and the overall stabilization of cardiac function [184]. However, it is interesting to note that upon the onset of heart failure, the proteasome itself is significantly down-regulated [113]. There has also been impaired ubiquitination and proteasomal degradation of proteins during chronic hypertrophy that could further accentuate damage caused by prior cardiac events [185]. In a long-term study evaluating pressure overload in dogs over 2 years, it was found that the upregulation of transcripts encoding various ubiquitin–proteasome proteins including poly-ubiquitin, processing proteins, and subunits occurred [169]. An upregulation of the proteasome noted in the middle stage of disease suggested a link between early-stage compensatory hypertrophy and mid-stage dilation; however, there is still debate as to whether the proteasome is up- or down-regulated in cardiac dysfunction [170, 171]. What has been observed in patients is an increase in poly-ubiquitinated proteins which could be a result of the UPS being overcome with superfluous poly-ubiquitinated proteins exceeding the degradative capacity of the proteasome and/or the possibility that there could be increased production of UPS substrates as a result of increased metabolic activity or misfolded proteins [155, 181–183]. The activity of the proteasome appears to vary among the distinct cardiomyopathies; therefore, when ruminating proteasome inhibition in an attempt to reduce excess protein degradation to impede remodeling, it is important to point out that its actions vary and inhibiting it in some cases, such as in hypertrophy, may accentuate damage to the myocardium instead of attenuating it. It is needed to further investigate the possibility of alternative proteasome subunit activity to determine its role in the pathogenesis of multiple diseases where the body is exposed to oxidative stress.

Although traditionally perceived as a degradative protease, calpain is also involved in cell apoptosis. It can act by cleaving BID which subsequently aids in releasing cytochrome *c* from the mitochondria to trigger intracellular apoptosis. Interestingly, this action has been demonstrated to be independent of the apoptotic caspase family [14]. Although predominantly a cytosolic protein, calpain has also been located in the mitochondria where it further contributes to cellular apoptosis. The increase in Ca^{2+} in the cell causes mitochondrial Ca^{2+}-overload which subsequently activates mitochondrial calpains to trigger apoptosis via cleavage of AIF [186–188]. AIF translocates to the nucleus to cause cell death independent of caspase activation and has been implied to occur in oxidative stress, hypoxia, and/or ischemia [187]. In addition to caspase-independent cell apoptosis, calpain has also been demonstrated to activate caspases, both directly and indirectly. Specifically, it can cleave apoptosis protease-activating factor-1 which, when combined with cytochrome *c*, activates caspase-9 in addition to directly converting procaspase-7 to its active form [188]. Calpain is closely correlated with MI as its ultrastructural degradative and pro-apoptotic nature is a primary culprit in the damage done to the heart as a result of oxidative stress and Ca^{2+}-overload. It is emphasized that the role of calpain in apoptosis in cardiomyopathies is yet to be determined, although its inhibition in cardiomyocytes subject to Ca^{2+}-overload could prevent both its proteolytic and apoptotic activities. Thus, it is evident that caspases are instrumental in both apoptosis and cardiac remodeling, and their inhibition may be pivotal in preventing both phenomena associated with cardiac dysfunction. In addition, degradation of proteins via the proteasome is another factor to consider regarding different cardiomyopathies as its activity appears to be up-regulated in cardiomyopathies leading to heart failure, but is then down-regulated as the disease processes. Finally, calpain is also associated with apoptosis as it activates AIF further contributing to cardiac dysfunction.

Concluding remarks

From the foregoing discussion, it is evident that different families of proteases are activated in the development of heart failure as a consequence of hypertension, cardiac hypertrophy, DCM, diabetic cardiomyopathy, and MI. The

activation of various proteases, including MMPs, calpains, cathepsins, and caspases, alters both the ECM and subcellular organelles and thus causes cardiac dysfunction. The mechanisms of protease activation involve oxidative stress and/or intracellular Ca^{2+}-overload as a consequence of elevated levels of some vasoactive hormones including catecholamines and the renin-angiotensin system in heart disease. The increase in proteolytic activity is also due to an imbalance in the activities of some proteases and their endogenous inhibitors such as TIMPs and calpastatin in the diseased myocardium. It has been observed that there occur differential changes in proteolytic activities where different proteases are activated at different stages of cardiovascular disease during the progression to heart failure. There are still large chasms in the knowledge regarding extracellular and intracellular proteolyic activities in both the ECM and subcellular remodeling processes for various cardiac pathologies and their collective progression toward heart failure. Finding common links would provide an ideal target and is a required step in developing therapies for the treatment of these distinct cardiovascular etiologies and prevent the progression to heart failure. Since different proteases are activated at a given stage of disease, it would be prudent to use combination therapy to inhibit more than one protease for the achievement of improved therapy of heart disease.

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