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Proteases in cardiometabolic diseases: Pathophysiology, molecular mechanisms and clinical applications

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Abstract

Cardiovascular disease is the leading cause of death in the U.S. and other developed country. Metabolic syndrome, including obesity, diabetes/insulin resistance, hypertension and dyslipidemia is major threat for public health in the modern society. It is well established that metabolic syndrome contributes to the development of cardiovascular disease collective called as cardiometabolic disease. Despite documented studies in the research field of cardiometabolic disease, the underlying mechanisms are far from clear. Proteases are enzymes that break down proteins, many of which have been implicated in various diseases including cardiac disease. Matrix metalloproteinase (MMP), calpain, cathepsin and caspase are among the major proteases involved in cardiac remodeling. Recent studies have also implicated proteases in the pathogenesis of cardiometabolic disease. Elevated expression and activities of proteases in atherosclerosis, coronary heart disease, obesity/insulin-associated heart disease as well as hypertensive heart disease have been documented. Furthermore, transgenic animals that are deficient in or overexpress proteases allow scientists to understand the causal relationship between proteases and cardiometabolic disease. Mechanistically, MMPs and cathepsins exert their effect on cardiometabolic diseases mainly through modifying the extracellular matrix. However, MMP and cathepsin are also reported to affect intracellular proteins, by which they contribute to the development of cardiometabolic diseases. On the other hand, activation of calpain and caspases has been shown to influence intracellular signaling cascade including the NF-κB and apoptosis pathways. Clinically, proteases are reported to function as biomarkers of cardiometabolic diseases. More importantly, the inhibitors of proteases are credited with beneficial cardiometabolic profile, although the exact molecular mechanisms underlying these salutary effects are still under investigation. A better understanding of the role of MMPs, cathepsins, calpains and caspases in cardiometabolic diseases process may yield novel therapeutic targets for threating or controlling these diseases.

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Keywords

Cardiometabolic diseases; MMP; calpain; cathepsin; caspase

1. Cardiometabolic diseases

Metabolic syndrome, a cluster of metabolic risk factors including obesity, diabetes/insulin resistance, hypertension as well as dyslipidemia, has been identified as a multiplex risk factor for cardiovascular disease [1, 2]. Although the diagnostic criteria for metabolic syndrome are still under debate, it is widely accepted that individuals with metabolic syndrome are at high risk for cardiovascular disease [3]. Cardiovascular disorders associated with metabolic syndrome are referred to as cardiometabolic diseases. Cardiometabolic diseases are multifactorial diseases with the involvement of a number of different factors including genetic, diets, lifestyle and living environment. Cardiac remodeling, coronary heart disease, even heart failure could result from metabolic syndrome. Along with the increased rates of obesity, diabetes and hypertension in the past decades, there has been an increase in the incidence of cardiometabolic diseases [4, 5]. Thus, recent research has targeted cardiometabolic diseases, with an aim to understand the pathogenesis of the disease and find potential clinical interventions to benefit subjects afflicted with these diseases. Recently, proteases have been implicated in the development and treatment of various disorders, especially cardiovascular disease. Given the increasing incidence of cardiometabolic diseases as well as the emerging role of proteases, this review summarizes the roles of major proteases including matrix metalloproteinase (MMP), calpain, cathepsin and caspase in cardiometabolic diseases.

2. Proteases

Proteins are the critical components for organisms and are involved in virtually all cellular functions. Besides protein production, the degradation of proteins is also important, as this is the way to recycle dysfunctional/damaged proteins and liberate the amino acids to form new proteins. Proteases are enzymes that perform protein catabolism by hydrolyzing peptide bonds that connects the amino acids to form the protein. To date, at least 500–600 proteases have been identified by using bioinformatic analysis [6]. Proteases are classified as serine, cysteine or threonine proteases, or as aspartic, –matrix metalloprotease and glutamic proteases based on their site of action [7]. Besides their traditional roles in protein turnover, proteases have been recently recognized as key - signaling molecules that participate in a number of vital physiological and pathological processes. The extracellular and intracellular protease including matrix metalloproteinase (MMP), calpain, cathepsin and caspase are among most extensively studied ones with respect to cardiovascular disease and remodeling. Research from our laboratory has focused on investigating the role of the cysteine proteinase cathepsin K in cardiometabolic diseases, including obesity, insulin resistance and hypertension-associated cardiac disease. Although emerging studies have shown the importance of proteases in cardiometabolic diseases, there are still some uncertainties remaining, and there is a paucity of review articles addressing the critical effects of proteases in cardiometabolic disease. Therefore, this review focuses on the role of proteases in the etiopathogenesis of cardiometabolic disease and attempts at addressing the potential

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molecular mechanisms involved in the process. To begin with, we shall briefly discuss the salient features of the various proteases that have documented role in cardiometabolic disease and subsequently address the role of each of these proteases in various disease conditions. In the subsequent section we shall address the potential clinical relevance of proteases and avenues for to harness them in the clinical setting, followed by a brief discussion on the future research questions which would further our understanding of the role of proteases in cardiometabolic disorders.

2.1. MMPs

MMPs are a class of metal-linked zinc-dependent proteases, whose biological activity requires calcium. MMPs cleave internal peptide bonds of proteins to degrade extracellular matrix (ECM). The MMP family is further classified as collagenases, gelatinases, stromelysins, elastases and membrane-type MMPs based on their enzyme characteristics. Collagenases, which include MMP-1, -8, and -13 cleave interstitial collagens I, II and III at specific sites and also cleave other ECM molecules such as gelatin and fibronectin. Collagen fragments are then degraded by gelatinases, which include MMP-2 and -9. Stromelysins, including MMP-3, -10 and -11 are responsible for gelatin, laminin and fibronectin degradation. Elastases, which include MMP-2, -9 and -12, degrade elastin in arterial wall [8, 9]. Membrane-type (MT)-MMPs are involved in the cleavage of types-I, -II and –III collagens and other components of ECM, which also activate proMMP to MMP [10]. The remarkable overlap in the activity of MMPs' and the preferred substrate despite their different protein structure, suggests redundancy. MMPs are synthesized as a proenzyme form followed by the hydrolysis of the zinc-cysteine bond to the mature form [11]. Vascular wall smooth muscle cells, endothelial cells, monocytes, macrophages, and T-cells have been shown to secrete MMPs [11]. The –expression of MMPs proenzymes is highly regulated by transcriptional mechanisms. Cytokines such as tumor necrosis factor-α (TNF-α) and interleukins are potent stimulants of the MMP proenzymes. Platelet-derived growth factor (PDGF) and CD40 ligands are reported to enhance MMP production as well [12]. In addition to regulation at the transcriptional level, the activity of MMP is elevated by oxygen free radicals, thrombin, chymase and angiotensin-converting enzyme (ACE) at post-transcriptional level [13]. Conversely, nature has designed endogenous MMP inhibitors [tissue inhibitors of metalloproteinases (TIMPs)] to counter-balance MMP activity. Four members of TIMP family are currently known, which include TIMP-1 to -4. TIMP-1 inhibits MMP-1, -3, -7 and -9. TIMP-2 inhibits MMP-2, whereas TIMP-3 is reported to decrease activities of MMP-2 and -9. TIMP-4 on the other hand inhibits MT-MMP and MMP-2 activity [14]. The exogenous inhibitors to MMPs, such as the tetracycline family of antibiotics are artificial MMP inhibitors that can blunt the activity of MMPs [15].

2.2. Calpains

The calcium ion-dependent papain-like protease (calpain) is a group of calcium-dependent, non-lysosomal neutral cysteine proteases [16, 17]. So far, at least 16 calpains have been identified, most of them, including calpain 1, requiring micromolar concentrations of calcium for activity. Interestingly, calpain 2 requires millimolar calcium concentrations. Calpains are ubiquitously expressed on all types of cells [18]. However, some calpains, such as calpain 3, which is a skeletal muscle-specific protease are tissue specific [19]. Localized

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in cytosol, calpains mainly target intracellular proteins. A large number of proteins have been reported to be degraded by calpains, which include, but not limited to Bax [20, 21], calcineurin [22], caspases [23], calmodulin-protein kinase [24], G protein [25], I_KB [26, 27], p53 [28, 29] and protein kinase C (PKC) [30, 31]. Although the amino acid sequences targeted by calpains ill-defined, it is widely accepted that amino acid sequence rich in proline, glutamic acid, serine and threonine elevate calpain-binding and calpain-dependent proteolysis [32]. Calpains are primarily produced and localized in the cytosol as proenzymes, which are then activated by intracellular calcium influx. Calcium binding relieves restrictions that are enforced by domain interactions and thus leads to activation of calpains [16]. Additionally, calpains are activated through direct phosphorylation at serine 50 by extracellular signal-regulated kinases (Erk) even without cytosolic calcium flux [33]. Calpastatin, the endogenous inhibitor of calpain tightly regulates the activity of calpains 1 and 2. The inhibitory effect is achieved by reversibly binding of calpastatin domains to calpain domains. Calpain activity can be inhibited through post-translational modification of phosphorylation as well [16]. In addition to the endogenous inhibitors, exogenous inhibitors of calpain, such as calpeptin have also been designed and characterized [34]. Interestingly, recent studies have shown that calpains are also secreted by a variety of cells (endothelial cells, lymphocytes, chondrocytes and osteoblasts) to extracellular space of tissues, which suggests a potential role of calpain in ECM degradation [35, 36].

2.3. Cathepsins

Cathepsins are a family of lysosomal proteases that were originally found in the gastric juice. So far, 19 cathepsins have been identified in mice [37]. They are classified into serine, aspartic and cysteine cathepsins according to the different catalytic activity. Cathepsins A and G are serine cathepsins, cathepsins D and E are aspartic cathepsins, whereas other cathepsins are cysteinyl cathepsins [38]. Although cathepsins were initially thought to function in acidic environment only, recent studies have found that they can be activated in neutral environment including cytosol [39, 40], nucleus [41] and even secretory vesicles [42, 43] as well. Similar to calpains, some cathepsins show tissue and cell-specific expression. For example, cathepsin K is highly expressed on bone tissue, especially the osteoclasts [44] whereas cathepsin S is primarily expressed on immune cells [45]. Unlike MMPs and calpains, cathepsins own a broad range of substrates that include almost all intracellular and extracellular proteins. Nonetheless, cathepsins prefer to degrade specific proteins, and therefore are implicated in specific physiologic process, including protein turnover in bone and cartilage [46], neuropeptide and hormone processing [47], antigen presentation [48] and apoptosis [49]. Recent studies confirm that cathepsins are synthesized as pro-cathepsins with an N-terminal signal peptide targeting ER proteins, followed by N-linked glycosylation [50]. Cathepsins are then bound to the mannose-6-phosphate receptor so as to localize them in the target lysosomes. The N-terminal peptides in pro-cathepsins are cleaved to activate the cathepsins [51, 52]. The activity of cathepsins is regulated by several factors, such as pH, oxidation and the presence of inhibitors. It is well known that cathepsins function optimally under slightly acidic environment with exceptions of cathepsin S and K which have been shown to be active under physiological pH [53]. Human cathepsins are activated under acidic conditions and are inactivated at neutral pH and under alkaline conditions [54]. However, it was reported that human cysteine protease cathepsin L was also inactivated at

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acid pH by a first-order process [55]. Reactive oxygen species (ROS) is a notorious stimulus for cathepsin release and activation [56, 57]. In addition, ROS-activated cytokines such as TNF-α, PDGF and IFN-γ also activate and secrete cathepsin B and L in fibroblast-like cells [58]. Similar results have been reported in neonatal cardiomyocytes, suggesting the potential role of cathepsins in cardiac diseases [59]. Additionally, angiotensin II (Ang II) is reported to enhance cathepsin gene expression in hearts [43]. Similar to MMPs and calpains, activities of cathepsins are inhibited by their endogenous and exogenous inhibitors. Cystatins are the natural inhibitors for cathepsins. Cystatins are classified into three groups based on the distinct structure, tissue/organ distribution and physiological functions. These include, stefins, cystatins and kininogens [60]. Cystatin reversibly binds to cysteine cathepsins [61]. In addition to natural inhibitors, artificial inhibitors to cathepsins, such as E64 have been designed for research and clinical use [62]. Among the synthetic cathepsin inhibitors, odanacatib, a cathepsin K inhibitor is currently under clinical trial to treat osteoporosis [63].

2.4. Caspases

Caspases are a family of acid proteases that use cysteine residues as the catalytic tools and cleave their substrates at the aspartic acid residues [64]. This family of proteases was first observed to be critical in the proteolytic maturation of IL-1β [65], until several years later, a pioneer study reported that these proteases play crucial roles in the execution of apoptosis [66]. Following this important finding, a number of members of the caspases family, such as caspase-1, -2, -3, -8 and -9 were identified to be crucial for apoptosis [67–73]. So far, 15 members of the caspase family have been discovered, which are essential components for the apoptotic machinery [74]. As reviewed before [75], capases (caspases-2, -8, -9 and -10) with a long pro-domain are called initiator of apoptosis caspases or group-II caspases. Conversely, caspases containing short pro-domains are named as executioner caspases or group-III caspases [76–78]. Together, these two groups of caspases play a critical role in regulating apoptosis - and are focus of discussion here. Substrate specificity of caspases has been described in great detail in previous reviews [75]. Four main substrate pockets of caspases named S1 to S4 have been reported. Caspases are ubiquitously expressed in all type of cells, which are initially translated from caspase genes as inactive pro-forms. Pro-caspases are originally synthesized as catalytically-dormant tripartite proenzymes with a single polypeptide chain of 32–55 kDa commonly representing 3 domains. Advanced protein structure techniques have identified N-terminal pro-domains of pro-caspases that contain motifs called death domain superfamily, which is essential for apoptotic signaling transduction [79, 80]. Pro-caspases are converted to their active forms by proteolytic processing at specific aspartic acid residues under the stimulation of a number of signals. Caspase activation occurs through autoactivation via oligomerization [81, 82], death receptor or mitochondrial pathways induced transactivation [83], as well as proteolysis by other proteases including caspases, cathepsins, calpain and granzyme B [84, 85]. The activated caspases subsequently initiate the apoptotic cascade or directly execute apoptosis to destroy DNA and the cell. Two classes of endogenous caspase inhibitors including natural caspase inhibitors and dominant negative caspases have been documented [86, 87]. Natural caspase inhibitors are derived from a wide spectrum of viruses, such as cowpox virus [88] and baculovirus [89]. Dominant negative caspases are the alternatively splicing of the

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primary transcript, which are enzymatically inactive and expresses as modified mRNAs or truncated proteins [86]. Peptide caspase inhibitors are synthetic peptide designed to target specific sequence of substrates that can be cleaved by other proteases [90, 91].

3. Role of proteases in cardiometabolic diseases

Several lines of evidence have suggested that proteases participate in the pathogenesis of varieties of diseases, such as cancer [92], inflammation [93, 94], neurodegenerative diseases [95, 96], liver diseases [97], chronic kidney disease [98] and cardiovascular disease [99, 100]. During the recent years, our understandings of the potential mechanisms by which proteases contribute to the development of cardiometabolic diseases have improved substantially. With this in mind, the involvements of MMP, calpain, cathepsin and caspase in cardiometabolic diseases and the potential molecular mechanisms involved in the process are reviewed below.

3.4. MMPs in cardiometabolic diseases

ECM is essential for the maintenance of structural integrity, cell anchoring, cell-cell communication, force transmission and for mediating cell survival/apoptosis and cardiac remodeling [101]. Interestingly, MMPs have been shown to were shown to induce the release of growth factors anchored in the extracellular matrix, consequently leading to cell proliferation, suggesting a potential mechanism by which MMPs induce cardiac hypertrophy and remodeling [102]. As the major class of proteases targeting the ECM, MMPs have been reported to be involved in the pathogenesis of several cardiovascular diseases associated with metabolic syndrome [14]. In fact, each component of the metabolic syndrome has been found to enhance MMP expression and/or activity, suggesting a pivotal role of MMPs in the etiopathology of metabolic syndrome and cardiometabolic disease [103–107]. The subsection below provides highlights of the role of MMPs in some of these conditions.

3.4.1. MMPs in atherosclerosis and coronary heart disease—Atherosclerosis and coronary heart disease are the most common cardiac disease in adults [108]. The atherosclerotic plaque is composed of a lipid core, comprising a mixture of inflammatory cells, especially foam cells and an integument of fibrous cap. The fibrous cap is mainly formed from the extracellular matrix laid down by the smooth muscle cells. Collagen and elastin are the major components for the fibrous cap. The fibrous cap is prone to destruction under the proinflammatory conditions, which results in the release of thrombus into circulation leading to thrombosis [109]. Coronary artery is the main vessel to be affected by atherosclerosis, and myocardial infarction and stroke are the major complications of atherosclerosis [110].

Numerous studies have reported elevated levels of MMPs in atherosclerotic plaques. Cytokines, such as TNF-α have been reported to induce MMP expression in smooth muscle cells through ERK-NF_KB signaling pathway [111]. MMPs were also activated by mast cell proteases in atherosclerotic plaques [112]. In animal models of atherosclerosis as well as human coronary specimens, colocalization of MMP-9 and -3 at the edges of atherosclerotic plaque has been demonstrated [113]. An *in vitro* study suggests enhanced MMP-2 and -9 mRNA levels in oxidized-low density lipoprotein (ox-LDL) loaded macrophages, which was

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inhibited by aspirin [114]. Almost all the MMPs are capable of interfering with the atherosclerotic plaque development and stability, which has been extensively reviewed in a recent review [115]. Interestingly, serum MMP-9 concentrations have been positively correlated to total carotid artery plaque score and instability, suggesting that MMP-9 could serve as potential marker for atherosclerosis [116]. Pro-inflammatory mediators and reactive oxygen species lead to the activation of MMPs, which further degrade collagen and elastin to weaken the fibrous cap and cause plaque rupture. The process is accelerated by mobilized macrophages and T cells localized in advancing zone of atherosclerotic plaque [117]. The creation of transgenic animal models with either overexpression or knockout of MMPs, further aided in the understanding of the role of MMPs in atherogenesis and coronary heart disease. One earlier study reported that MMP-1 and ApoE double knockout mice transgenic for human MMP-1 gene in macrophages, exhibited smaller plaques with less collagen [118]. In contrast, overexpression of an auto-activated MMP-9 led to high levels of plaque instability in the same mouse model [119]. Similarly, overexpression of MMP-12 in rabbits enhanced plaque size and inflammation [120]. It was recently found that MMP-9 and ApoE double knockout mice displayed reduced atherosclerotic load despite being fed with a cholesterol rich food [121]. Similar results were obtained when using a MMP-2 and ApoE double knockout mouse model [122]. In contrast however, MMP-3 and ApoE double knockout mice showed increased plaque size [123]. Elevated level of MMP-9 in subjects with coronary artery disease with unstable angina has also been reported [124]. Thus, although the exact role of MMPs in atherosclerosis is remains controversial, based on the aforementioned studies it can be concluded that elevated levels of MMPs, especially MMP-2 and -9 is detrimental, in that, they enhance matrix destruction and cause inflammation, which possibly results in plaque rupture.

Endothelial erosion that occur in highly stenotic, fibrotic plaques without the presence of inflammation has been postulated to play a predominant role in the loss of endothelial cells, which is the other common trigger for coronary heart disease [125]. MMPs are expressed on the endothelial cells which express a variety of MMP substrates (including urokinase-type plasminogen activator receptor and TGF-beta) and activators such as tissue plasminogen activator [126–128]. Endothelial MMPs are also activated by cytokines including TNFα and IL-1 [129, 130]. Furthermore, endothelial MMP-9 expression can be up-regulated by oscillatory flow through activation of c-myc [131]. The elevated endothelial MMPs expression and activation may thus contribute to the development and progression of atherosclerosis and coronary heart disease.

It is well known that angiogenesis, the formation of new microvessels, occurs in atherosclerotic plaques leading to neovascularization and the growth of the lesion [132]. Although the mechanisms of involved in this process are not fully understood, MMPs, especially MMP-2 and -9, have shown to play a pivotal role in the process of angiogenesis. Elevated MMP-2 and -9 were reported in arteries from subjects with diabetic chronic kidney disease, which correlated to impaired angiogenesis and endothelial dysfunction [133]. A recent study demonstrated that MMP-8 plays an important role in angiogenesis as well. In this study, Fang and coworkers demonstrated that MMP-8/apoE double knockout mice displayed attenuated angiogenesis and smaller atherosclerotic plaque size compared to the apoE knockout mice, concluding that MMP- 8 triggers atherosclerotic formation, and

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targeting MMP-8 is as a potential therapeutic target of atherosclerosis [134]. In fact, numerous angiogenic signaling pathways, such as those that are induced by the growth factors FGF and VEGF have been shown to upregulate MMPs via Erk, JNK and Akt activation, which further consolidate the evidence that MMP-associated angiogenesis contribute to the development of atherosclerosis [135–137].

3.4.2. MMPs in obesity and insulin resistance-associated cardiac disease— Obesity, which is defined as an increased body mass index (> 30 kg/m^2), is an important health issue for Americans in view of its increasing prevalence [138]. Obesity usually accompanies with insulin resistance, which is the fundamental pathological change that predisposes to type 2 diabetes. Both obesity and insulin resistance are important independent risk factors for cardiac disease [139]. Several mechanisms contribute to obesity and/or insulin resistance-induced cardiac disease although the exact mechanism involved is far from clear [140]. Interestingly, elevated plasma MMP-2 and -9 levels have been found in obese women and children [141, 142], which imply that MMPs are potentially involved in obesity-associated heart disease. This notion received support from the evidence of an animal study published in 2001 by Peterson and coworkers who found that the MMP-2 activity in the left ventricle was elevated in obese, spontaneously hypertensive heart failure (SHHF) rats, which paralleled impaired ventricular function. More importantly, pharmacological inhibition of MMP alleviated left ventricle dysfunction and remodeling in SHHF rats, suggesting that MMP activity contributed to left ventricle dysfunction in obese animals [143]. Sucrose-enriched diet feeding resulted in lipid accumulation accompanied by upregulation of cardiac MMP-2, MMP-9 and TIMP-1 expression in rats, which were reversed by L-arginine supplementation [144]. Another study showed that the activity of MMP-7, a critical regulator of cardiac fibrosis was increased in the diabetic heart as well [145]. Conflicting results were however found in a human study, in which authors demonstrated that abnormalities of left ventricle function in premenopausal obese women were related to the decreased plasma MMP-2 levels, possibly because the suppressed MMP system attenuates ECM degradation [146]. Results from an animal study further revealed that MMP-2 activity was decreased in a rat model that spontaneously develops diabetes mellitus. In this study, the authors observed that angiotensin II receptor blockade prevents left ventricular diastolic dysfunction by restoring MMP-2 activity [147]. Further research is necessary to ascertain the role of MMPs in obesity and insulin resistance-associated cardiac disease.

3.4.3. MMPs in hypertensive heart disease—Normal systolic blood pressure is within the range of 100–140 mmHg, whereas diastolic blood pressure is within the range of 60–90 mmHg. Hypertension is defined as a blood pressure that is persistent at or above 140/90 mmHg. Untreated or uncontrolled hypertension is an important risk factor for cardiac disease, which is called hypertensive heart disease. The role of MMPs has been extensively studied in hypertensive heart disease due to the involvement of ECM in cardiac remodeling that is commonly associated with the hypertensive heart. Elevated MMPs expression levels have been documented in hearts from different models of hypertensive animal, including angiotensin II-induced hypertension [148], pulmonary hypertension [149], renovascular hypertension [150], spontaneously hypertensive rats [151], volume overload [152] and

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pressure overload-induced hypertension [153]. Studies by using transgenic models further declare the contributions of MMP/TIMP in hypertensive heart disease. Overexpression of MMP-1 has been reported to correct the accumulation of cardiac fibrosis, cardiac dysfunction and remodeling in response to chronic pressure overload [154]. Conversely, MMP-2 knockout mouse was resistant to pressure overload-induced left ventricular hypertrophy and dysfunction [155]. MMP-7 inhibition by genetic knockout and pharmacological inhibitors displayed attenuated hypertrophy through correction of a disintegrin and metalloproteinase-12 (ADAM-12) overexpression [156]. Furthermore, angiotensin-converting enzyme inhibitor was shown to block MMP activity and thus protected left ventricle from remodeling and dysfunction [157]. Additionally, MMP-associated angiogenesis has been documented to be crucial in the transition from compensatory hypertrophic heart to decompensatory failing heart. In this study, increased MMP-2 as well as increased angiogenic factors were observed in hypertrophic heart, whereas an increase in MMP-9 and angiostatin were found in failing heart [158].

3.5. Calpains in cardiometabolic diseases

As discussed under section 2.2., calpain targets a wide spectrum of proteins, suggesting a key role of calpain in cardiac diseases. Accordingly, the involvement of calpain in cardiometabolic diseases has been well documented. The following section briefly discusses the role of calpain in various cardiometabolic diseases.

3.5.1. Calpain in atherosclerosis and coronary heart disease—It is widely accepted that calpains act in the endothelial cells and helps maintain vascular integrity. A number of factors such as VEGF and shear stress activate the calpain system under physiological condition [159, 160]. Mechanistically, calpain is able to regulate small GTPase [161] and lyse focal adhesion proteins [160]. Interestingly, it has been documented that nitric oxide production is mediated by calpain-induced proteolysis of HSP90 or PI3K/AMPK signaling cascade [159, 162]. In addition to its physiological role, calpain participates in the pathogenesis of various diseases. An earlier study revealed that $sPLA_2$-modified LDL or oxidized LDL enhanced m-calpain expression in endothelial cells on atheroma [163, 164]. Activated calpain directly cleavages VE-cadherin and thus facilitates the extravasation of inflammatory cells and macromolecules into the vascular wall [165]. Silencing *CAPN2* by siRNA technique further confirmed that knockdown of calpain induces pro-atherogenic hyperpermeability in the murine aorta [163]. It has been reported that calpain activity is also enhanced in endothelial cells from lipopolysaccharide-accelerated atherosclerosis [166]. Cytokines and inflammatory signals are involved into the development of atherosclerosis. Nuclear factor-κB (NF-κB) signal, a representative inflammatory signal, was modulated by calpain via degradation of IκB without affecting its phosphorylation [167]. Not surprisingly, NF-κB as well as cytokines levels in endothelial cells from pro-atherogenic aorta in $LDLr^{-/-}$ mice were remarkably decreased following the administration of calpain inhibitors [163]. Macrophage-derived foam cells are hallmark for atherosclerosis. Activated ATP-binding cassette transporter A1 (ABCA1) in macrophages is shown to be dampened by calpain-induced proteolysis [168]. Calpain has also been shown to interfere with cholesterol efflux from macrophage through the degradation of the ATP-binding cassette transporter G1 (ABCG1) [169]. In smooth muscle cells, activation of

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calpain was reported to precede the activation of caspase in response to degraded collagen, whereas, inhibition of calpain was observed to reduce apoptotic response, suggesting a potential role of calpain in atherosclerotic plaque rupture [170]. Evidence from a clinical study confirmed the association of calpain-10 with atherosclerosis and coronary heart disease in human [171].

3.5.2. Calpain in obesity and insulin resistance-associated cardiac disease— *CAPN10* has been identified as a diabetic gene and a number of positional cloning studies have identified that genetic variation in *CAPN10* accounted for 14% of the population-attributable risk to type 2 diabetes in Mexican Americans [172]. Several studies published subsequently, using a wide range of ethnic populations further confirmed the association of *CAPN10* with type 2 diabetes [173–178]. This suggests that calpains may be potentially involved in insulin regulated pathway. The first paper in support of the role of calpain on insulin signaling published in 1990s, demonstrated that calpain regulates the expression of insulin receptor substrate-1 (IRS-1) [179]. A series of recent studies strongly support this notion that calpain regulates insulin signaling pathway. Calpain inhibitors prevented IRS-1 downregulation, while drug-induced overload of intracellular Ca^{2+} could restore the suppressed IRS-1 level [16]. A clinical study demonstrated that activation of calpain was detrimental to the diabetic myocardium, and blocking calpain activation protected heart from diabetes-associated cardiac injury [180]. Nonetheless, literature regarding the contribution of calpain in obesity, insulin resistance and associated cardiac disease are limited warranting further studies to understand the exact nature of the role of calpains in obesity and insulin resistance-associated cardiac disease.

3.5.3. Calpain in hypertensive heart disease—Several transcriptional factors, such as NF-κB, GATA binding protein 4 (GATA4) and nuclear factor of activated T cells (NFAT) participate in the development of hypertension-induced cardiac hypertrophy. Interestingly, calpain has been reported as a critical regulator for NF-κB, GATA4 and NFAT, implying the possible role of calpain in cardiac hypertrophy [167, 181, 182]. Enhanced calpain activity as well as decreased calpastatin expression was found in hearts from mice subjected to angiotensin II infusion [183]. In the same study, experiments performed on transgenic mice that constitutively express calpastatin revealed that infusion of angiotensin II failed to induce cardiac hypertrophy, although those mice did develop hypertension. Parallel to this finding, angiotensin II-induced NF-κB over-expression was blunted by calpastatin transgene in mice, which may represent the molecular mechanism by which calpastatin overexpressed mice exhibited resistance to hypertension-associated cardiac hypertrophy [183]. In line with these findings, pre-administration of calpeptin, a specific calpain inhibitor, to hypertensive β3-integrin-deficient mice alleviated cardiomyocyte apoptosis and prevented cardiac hypertrophy [184]. Another study suggested that elevated calpain activation in hypertrophic heart could lead to the degradation of focal adhesion kinase as well as calcineurin to worsen cardiac hypertrophic response [185]. Treatment with calpain inhibitor attenuated cardiac hypertrophy via inhibiting the degradation of these proteins. Indeed, most substrates of calpain are potentially involved in the pathogenesis of hypertensive heart disease. However, more conclusive studies are necessary to evaluate the molecular pathways by which calpains contribute to cardiac hypertrophy.

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3.6. Cathepsins in cardiometabolic diseases

Studies in the recent years have implicated a role of cathepsins in the etiopathology of cardiometabolic disease. Several cathepsins, such as cathepsin S, K, B and L have been found to be expressed in cardiomyocyte, cultured cardiac fibroblasts and/or myofibroblasts [59, 186, 187]. Although the expression of cathepsins in heart is negligible at basal conditions, stimulation by cytokines, angiotensin II and superoxide can remarkably enhance their expression [43]. The following section provides an outline of our understanding of the role of cathepsin in various cardiometabolic diseases.

3.6.1. Cathepsins in atherosclerosis and coronary heart disease—Cathepsins exhibit potent collagenase and elastinase activity, by virtue of which they potentially participate in the formation and rupture of atheroma. Early studies documented that increased cathepsin activity was associated with experimental atherosclerosis [188]. Two decades ago, Reddy and the colleague observed that human macrophages secrete the active form of cathepsin S, B and L [189]. Subsequent studies showed that cathepsin D regulates ABCA1-mediated lipid efflux and decreased levels of cathepsin D levels results in low plasma HDL-C levels [190]. Platt and coworkers reported an increased expression of cathepsin K in the endothelium of human subjects with atherosclerosis [191]. Consistent with these observations, cathepsin levels were also found to be elevated in a diet-induced animal model of atherosclerosis [192]. A recent study investigated the relative expression of cathepsins at different sites of atherosclerotic plaque. Increased cathepsin expression was detected in sites prone to rupture, including macrophages bordering the lipid core and adjacent to the fibrous cap or macrophages/smooth muscle cells in the shoulder regions [193, 194]. Cathepsin L has been shown to be the major contributor to apoptosis of the macrophages resulting in necrotic core formation, leading to atherosclerotic plaque instability [195]. Interestingly, one recent study showed that cathepsin G deficiency attenuated the complexity of atherosclerotic lesions in apolipoprotein E-deficient mice via dampening apoptosis [196]. Cathepsin S, on the other hand, improves fibrous cap stabilization, and helps monocyte adhesion and migration in an *in vitro* system [197]. Cathepsin K levels were found positively correlated to the percent plaque volumes and negatively correlated to percent fibrous volumes. Additionally, cathepsin K blood levels have been claimed to be an independent predictor of coronary heart disease [198]. Similarly, serum cathepsin L contents were significantly increased in patients with coronary heart disease [199, 200]. However, Mirzaii-Dizgah and coworkers failed to observe any changes in serum and salivary cathepsin L levels in subjects with coronary heart disease [201]. Cystatin c, an endogenous inhibitor of cathepsin, has also been implicated in the development of coronary heart disease in a human study. The mutant haplotype of cystatin c gene was related to higher average number of stenosis per coronary artery segment [202]. Collectively these studies show that cathepsins contribute to the development of atherosclerosis and coronary heart disease. However different cathepsins may have different role in the pathogenesis – some of them being detrimental whereas some others being protective. Targeting cathepsins my thus represent an attractive strategy to treat atherosclerosis and coronary heart disease.

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3.6.2. Cathepsins in obesity and insulin resistance-associated cardiac disease—In the year 2003, cathepsin K was identified as a novel marker of adiposity in white adipose tissue owing to its high levels of expression in white adipose tissue in the *db/db* mice [203]. Moreover, the expression of the transcription factors involved in the induction of cathepsin K were also elevated in the white adipose tissue from obese mice [203]. Subsequent studies ascertained the role of cathepsin K in adipocyte differentiation. Inhibition of cathepsin K by either pharmacological inhibitor or genetic ablation was sufficient to reverse adipocyte differentiation and lipid accumulation in response to high-fat diet feeding [204, 205]. Cathepsin K inhibition prevented high-fat diet or leptin deficiency-induced obesity as well as elevated serum glucose and insulin levels by degrading fibronectin [206]. Degradation of type I collagen has also been proposed as a potential mechanism by which cathepsin K participates in adipogenesis during the early differentiation phases [207]. Cathepsin S is another extensively studied cathepsin in terms of its role in the obesity. A number of studies have reported elevated levels of cathepsin S in adipose tissues from both human and animal models [208–210]. Interestingly, weight loss in morbidly obese women resulted in reduced expression of cathepsin S in the adipose tissue and lower circulating levels of this protease [211]. Recently, cathepsin D was also shown to be up-regulated in obese mouse and human adipose tissue [212]. In addition, activated cathepsin D associated with adipocyte hypertrophy triggered the activation of proapoptotic proteins [213].

Expression levels of cathepsin L in the muscle has been shown to be elevated in glucose intolerant mice [214]. In the skeletal muscle of diabetic subjects, reduced insulin-stimulated cathepsin L gene expression was also reported in this paper, suggesting that impaired cathepsin L expression is secondary to impaired glucose metabolism [214]. Cathepsin S levels strongly correlate with insulin resistance as reported by Jobs and coworkers, who in their study showed that subjects with higher cathepsin S levels had decreased insulin sensitivity and higher risk to develop type 2 diabetes [215]. This finding was further supported by a study from Chen and colleagues who showed a correlation between serum cathepsin S and insulin resistance, in type 2 diabetic subjects [216].

In our lab, we investigated the role of cathepsin K in obesity-associated cardiac dysfunction. We found that genetic ablation of cathepsin K in mice protected hearts from high-fat diet feeding-induced geometric and functional impairment, as evidenced by recovered fractional shortening as well as peak shortening of single cardiomyocytes. Mechanistically, insulin signaling pathway was dampened in the heart from high-fat diet feed mice, which was rescued by cathepsin K knockout. Moreover, activated apoptosis in mouse heart consequent to high-fat diet feeding was attenuated by cathepsin K knockout [187]. These studies substantiate the notion that cathepsin K participates in obesity-associated heart disease and targeting cathepsin K maybe a promising approach to counter obesity-associated complications in the heart.

3.6.3. Cathepsins in hypertensive heart disease—The early report linking cathepsin to hypertension was from a study by Saito and coworkers, wherein an increased cathepsin level in the serum of spontaneously hypertensive rats was observed [217]. A study published in the following year further by Wildenthal and coworkers confirmed the role of this

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lysosomal enzyme in the development of hypertension-induced cardiac hypertrophy [218], which was substantiated by another study by Rozek and colleagues [219]. Impaired cathepsin B activity was displayed in hypertensive heart, which was reversed by the treatment with ACE inhibitor. Additionally, the ACE inhibitor alleviated hypertension-associated cardiac hypertrophy in rats [220]. Cheng and coworkers investigated the role of cathepsin in hypertension-induced heart failure in rat [59]. They identified cathepsin S as the predominant cathepsin that is elevated in the failing heart. Furthermore, the expression and activity of cathepsin S was induced by IL-1β in cultured neonatal cardiomyocytes, suggesting that inflammatory mediators activate cathepsin S. A follow-up study from same group reported that superoxide anions activate cathepsin, which in turn triggers myocardial remodeling [43]. Blocking angiotensin II type 1 receptor attenuated myocardial remodeling and dysfunction via blunting superoxide-dependent induction of cathepsin. Additionally, the involvement of cathepsin G in the alternative pathways of angiotensin II biosynthesis makes cathepsin G a potential mediator in the development of hypertensive heart disease, although studies to this effect are lacking [221].

Despite the documented role of cathepsins in the pathophysiology of hypertensive heart disease, the underlying molecular mechanisms remain elusive. Most effects attributed to cathepsins in hypertensive heart disease are linked to the degradation of collagen or elastin. However, recent studies have suggested that cathepsins are involved in the crosstalk with several signaling pathways, which may contribute to their observed effects. The Akt/GSK3β pathways have been extensively studied in cardiac hypertrophy because of their effects on inflammation, fibrosis as well as apoptosis. By using a cathepsin L overexpressed mouse model, Tang and coworkers showed that cathepsin L protects against hypertension-induced cardiac hypertrophy via inactivation of the Akt/GSK3β signaling pathway [222]. In contrast, cathepsin L knockout in mice was shown to accentuate pressure overload-induced cardiac hypertrophy [223]. Impaired lysosomal protein degradation, increased sarcomere-associated protein aggregation, increased ubiquitin-proteasome system as well as altered endoplasmic reticulum homeostasis were found in hypertensive cathepsin L knockout mice, all of which resulted in the worsening of cardiac function and cardiac remodeling in response to pressure overload. We evaluated the role of cathepsin K knockout in mice subjected to pressure overload-induced cardiac hypertrophy [224]. Our results support the notion that cathepsin K knockout in mice prevents the development of cardiac hypertrophy and contractile dysfunction. Moreover, the stimulation of the mTOR and Erk signaling pathways which were induced in hypertrophic heart, were blunted by cathepsin K knockout and in cultured cardiomyocytes subjected to siRNA mediated silencing of cathepsin K. Furthermore, plasmid-mediated overexpression of cathepsin K in the cultured cardiomyocytes triggered cardiomyocyte hypertrophy, which was blocked by mTOR and Erk inhibitors. Collectively, our data suggest the important role of cathepsin K in cardiac hypertrophy induced by hypertension.

3.7. Caspases in cardiometabolic diseases

Caspases are essential players in the apoptotic cascade, playing a pivotal role in the initiation and execution of apoptosis. Volumes of literature have reported the involvement of apoptosis in cardiac disease, including cardiometabolic disease and a detailed discussion is

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beyond the scope of this review. The role of caspase in cardiometabolic disease will be the focus here.

3.7.1. Caspases in atherosclerosis and coronary heart disease—Macrophages are the most abundant inflammatory cells in atherosclerotic plaque. Macrophage-derived foam cells with lipid core represent the major component of the atherosclerotic plaque. Fas or other death receptor triggered apoptosis has been proposed to contribute to plaque macrophage death and even plaque development [225, 226]. Importantly, expression of c-FLIP, a dominant-negative inhibitor of caspase-8, has been reported to protect plaque macrophages from apoptotic cell death [227]. Role of caspase in macrophage apoptosis has been extensively reviewed in a previous literature [228]. Oxidized LDL and cholesterol accumulation participate in plaque cell death [226, 229]. Oxidized LDL-induced apoptosis involves both the death receptor and mitochondrial apoptotic pathways. Caspase cascade is in turn activated, which triggers apoptosis [230]. Besides macrophage, vascular cells such as endothelial cells and smooth muscle cells undergo apoptosis in atherosclerosis plaque as well. The role of apoptosis in plaque endothelial cells and smooth muscle cells has been reviewed elaborately in previous reports [231, 232].

3.7.2. Caspases in obesity and insulin resistance-associated cardiac disease—Lipoapoptosis has been observed in obesity and type 2 diabetes, which is an important feature that contributes to obesity-associated cardiac dysfunction. Accumulated unoxidized fatty acids in obese subjects trigger toxic pathways, such as ceramide pathway to initiate apoptosis in cardiomyocytes [233]. Increased cardiomyocyte fatty acid oxidation as well as inhibition of glucose uptake have been reported to induce apoptosis and result in dilated cardiomyopathy in mice expressing glycosylated-inositol (GPI)-anchored human lipoprotein lipase (hLpLGPI) [234]. Studies from our group have documented the involvement of caspase activation and apoptosis in cardiac dysfunction in response to high-fat diet feeding [187, 235]. Additionally, in genetically obese mice, enhanced apoptotic response in cardiomyocyte was accompanied by increased DNA damage and decreased survival rate [236]. Elevated cardiac Fas receptor-dependent apoptotic pathway has been observed in obese Zucker rats [237].. Increased protein levels of Fas ligand, Fas death receptors, Fas-associated death domain were significantly upregulated in obese rat hearts, which was paralleled with enhanced caspase-8 and -3 activities, suggesting the involvement of Fas receptor-dependent apoptosis in obesity-associated heart disease [237]. Interestingly, another study from the same group reported activated cardiac mitochondrial-dependent apoptotic pathway in obese Zucker rats. Increased Bcl-2/adenovirus E1B 19 kDa interacting protein (BNIP3), Bad expression as well as cytochrome c release were observed in hearts from obese rats. A reciprocal suppression of the antiapoptotic Bcl2 protein level was observed. Moreover, an increase in the expression of activated caspase-9 and -3 were reported, suggesting the involvement of mitochondrial-dependent apoptotic pathway in obesity-associated heart disease [238]. A follow-up study further confirmed the effect of mitochondrial apoptotic pathway in obesity-associated cardiac disease. Furthermore, aerobic exercise training was effective in blocking cardiac mitochondrial apoptotic signaling and rescuing cardiac dysfunction [239]. More importantly, reduction in body mass resulted in attenuated apoptosis, oxidative stress and inflammation, which rescued left ventricular

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remodeling as well as heart dysfunction in obese mice [240]. Cardiac remodeling caused by a high-carbohydrate, high-fat diet-induced metabolic change was alleviated by rutin via its antiapoptotic properties [241]. Collectively, the aforementioned studies provide strong evidence in support of the relationship between caspase-induced apoptosis and obesity/insulin resistance-induced heart disease.

3.7.3. Caspase in hypertensive heart disease—Ravassa and the colleagues reported the contribution of apoptosis in angiotensin II-induced ventricular cardiomyocyte dysfunction in the spontaneously hypertensive rats [242]. This finding was supported by the studies from the labs of Lopez-Farre and [243] and deBlois group [244]. Interestingly, the elevated apoptosis in hypertrophic hearts of spontaneously hypertensive rats was attenuated by exercise training [245]. Moreover, cardiomyocyte apoptosis was triggered in the failing hearts of hypertensive human subjects. On the other hand, the expression of gp130, a protein preventing cardiomyocyte apoptosis, was decreased in failing heart of hypertensive patients. There was a negative correlation between gp130 and cardiomyocyte apoptosis in hypertensive patients that develop heart failure [246]. Endoplasmic reticulum (ER) stress-induced apoptosis was reported in the hypertensive heart in high-salt diet fed Dahl salt-sensitive rats. Caspase-12 has been shown to be involved in the ER stress-associated cardiomyocyte apoptosis in hypertensive heart [247]. Interestingly, caspase-3 and calpain activities were elevated at the same time in the pressure overload-induced hypertrophic heart, whereas calpain inhibitor could block caspase-3 activation and prevent cardiac dysfunction. However, caspase inhibitor was not beneficial to cardiac function in the pressure overload model. Based on these findings the authors concluded that calpain may be the key mediator of cardiomyocyte death, although apoptosis cascade may be involved in the progression of end-stage hypertrophy to heart failure [34]. The exact role of caspase and apoptosis in hypertensive heart disease remains unclear although the activation of apoptosis under this condition is definite.

4. Potential clinical application of proteases

Due to the important role of proteases in ECM degradation, intracellular protein degradation and participation in a variety of singling pathways, it is reasonable to expect the possibility of harnessing proteases and their endogenous and synthetic inhibitors in for the diagnosis or treatment of cardiometabolic diseases. MMPs, cathepsins and caspases have been suggested as potential diagnostic markers for the detection and prognosis of cardiometabolic disease. Recent studies have shown that elevated levels of serum MMP-9 was found in atherosclerotic carotid artery, and is related to the plaque vulnerability, suggesting that cathepsins can also be potential markers of cardiovascular disease [248]. In another study, Shirakabe and coworkers investigated the change of serum MMP-2 levels in subjects with acute heart failure before and after treatment and its relationship to clinical prognosis was investigated. Following treatment for acute heart failure, the patients had decreased levels of MMP-2, indicating that prognosis of heart failure was markedly better among the patients with a altered MMP-2 levels [249]. Gene polymorphism of MMP-9 was found to increase the risk of clinical events in coronary heart disease patients. Human subjects with coronary artery disease exhibiting IL-18+183 AA/MMP-9-1562 CT/TT combined genotypes were shown to have a higher risk of clinical events [250]. In contrast, patients with 6A-1171-

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G-519 haplotype had a reduced risk of clinical events [251], suggesting that genotyping for MMP may represent a diagnostic tool for cardiac health. Cathepsins have also been suggested as potential biomarkers for cardiometabolic diseases [200]. Serum cathepsin L levels were higher in patients with acute myocardial infarction compared to those with unstable angina pectoris. Additionally, serum cathepsin L levels were positively related to number of coronary branch luminal narrowing and Gensini scores. Furthermore, cathepsin L levels have been negatively correlated to high-density lipoprotein and apolipoprotein, suggesting the potential diagnostic value of serum cathepsin L levels in coronary heart disease [200]. Cystatin C, the endogenous inhibitor of cathepsin, has also received attention as a biomarker for cardiometabolic diseases, including atherosclerosis [252], heart failure [253] and hypertensive cardiac hypertrophy [254]. As the direct executioner of apoptosis, caspases have also been evaluated as a potential biomarker of cardiometabolic diseases. Elevated serum caspase-1 content was observed in patients with acute angina [255]. Circulating caspase-3 p17 level has also been identified as a potential biomarker for cardiovascular disease [256].

During the past decade, several pharmaceutical companies have been engaged in designing and developing new drugs to target proteases for the treatment of cardiometabolic diseases. Two MMP inhibitors, KB-R7785 from Kanebo KK Company and SC-44463 from Pfizer are being developed for the treatment of cardiovascular disease [257]. Additionally, some widely used drugs like doxycycline has been tested for its efficacy in the treatment of coronary heart disease, by virtue of its effect on MMP [258]. Among the cathepsin family, pharmacological inhibition of cathepsin K has garnered the most interest because of its significant role in bone resorption. Currently, there are three cathepsin K inhibitors under clinical trial for treatment of osteoporosis. One such cathepsin K inhibitor Odanacitib, which is effective and without major side effects (following a 5-year trial), is currently under phase III clinical trials [259]. In view of the cardio-protective role of cathepsin K inhibition as evidenced by our lab and that of others, we can speculate the potential application of cathepsin K specific inhibitors in treating cardiovascular disease. Various caspase inhibitors have been designed to target a wide spectrum of diseases. Among the caspase inhibitors, pan-caspase inhibitors, IDN- 6734 and MX1013 have been tested [260].

5. Where are we in our understanding of the role of proteases in cardiometabolic disorders?

Although there are numerous proteases that have been identified the key proteases with demonstrated role in cardiometabolic diseases include the MMPs, calpains, cathepsins and caspases and their endogenous activators and inhibitors. There are a number of subtypes within these proteases, some of which have similar or opposing actions suggesting redundancy and counterregulatory functions. Despite growing evidence supporting a functional association between proteases and cardiometabolic diseases, it yet unclear if the upregulation of protease activity represent a cause or consequence (or both) of cardiometabolic disorders. It is likely that inflammatory mediators such as reactive oxygen species and cytokines activate proteases which can lead to proteolysis of substrates involved in key pathways regulating a variety of cardiometabolic functions. It is also likely that

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activated proteases upregulate inflammatory mediators resulting in a vicious cycle. Thus, the emerging consensus is that proteases play a critical role in cardiometabolic disease and targeting proteases may represent an important therapeutic strategy.

The proteases discussed above have all been strongly implicated in the progression of diabetic cardiac disease and heart failure. Among the proteases described above MMPs have been extensively studied for their role in diabetes induced cardiac remodeling and dysfunction. MMPs play a critical role in the cardiac remodeling event which is characterized by synthesis and degradation of extracellular matrix. Thus for MMPs, the etiopathology of cardiometabolic diseases may be attributed to their traditional proteolytic processing of signaling molecules. Whereas targeting of MMP activation is therefore a potential clinical strategy to treat cardiometabolic disease, altering TIMP may also serve as an important strategy to counterbalance the effects of MMP. On the other hand, being a calcium-activated protease calpain plays a major role in regulating calcium-regulated cellular process such as cardiac contractility, hypertrophy and apoptosis. However, calpains have also been shown to be important for maintaining protein homeostasis in the cardiac cells, and therefore a deficiency of calpain may also prove to be detrimental. This dichotomy suggests that calpains serve a regulatory role; whereas excessive calpain activity needs to be curbed, low levels of calpain activity may have to be boosted. Further studies are necessary to distinguish these diverse roles of calpain and to ascertain conditions wherein one strategy supersedes another. At the molecular level, calpains are thought to mediate their detrimental cardiovascular via the activation of NF_KB and the tumor TGF-beta signaling pathways. Calpains also play a critical role in cell death via activating the apoptosis pathway and/or proteolytic degradation of molecules involved in the apoptotic pathway. As discussed above, variation in calpain-10 gene has been shown to be associated with diabetes. However, understanding the implication of this genetic variation in cardiometabolic disease is a matter of great interest. Cathepsins on the other hand are recent entrants in the field. Among the cathepsins, the cysteinyl cathepsins that localize in the endosomes or lysosomes are thought to play a major role in cardiometabolic disease as lysosomal membrane instability caused by a variety of cellular stressors can lead to the release of these proteases. Although these proteases are optimally active in the acidic environment some of them may retain their activity in the neutral cytoplasmic or extracellular environment and mediate their potent elastolytic or collagenolytic activity. Caspases on the other hand have been shown to mediate apoptosis. MMPs, calpains and cathepsins can cause proteolytic activation of caspases leading to cell cellular apoptosis suggesting a cross-talk between different proteases.

Future research should be directed at studying the global changes in these proteases to address the following questions i) Is there a redundancy and counter-regulation among the proteases; and among proteases and their respective inhibitors? ii) In addition to their proteolytic activities are non-traditional mechanisms (such as autophagy), involved in mediating the activities attributed to these proteases? iii) What is the extent of cross-talk between these proteases? iv) What are the upstream regulators and downstream effectors of proteases, and can any of these be used as biomarkers of cardiometabolic syndrome and/or targets for treatment? Investigating these and other relevant questions would provide useful insights to our growing understanding of the role of proteases in cardiometabolic disease.

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6. Conclusion

Emerging studies suggest a strong role of proteases especially MMPs, calpain, cathepsins and caspases in the development of cardiometabolic diseases, including atherosclerosis/coronary heart disease, obesity/insulin resistance-associated heart disease and hypertensive heart disease. Targeting these proteases would therefore represent a novel therapeutic approach for treating or controlling cardiometabolic diseases. Studies in human and animal models show that the expression of MMPs, calpain, cathepsins and caspases under cardiometabolic conditions are regulated by a variety of stimulants such as cytokines, ROS and angiotensin II. Traditionally, the activated proteases either degrade ECM or intracellular proteins to contribute to the pathogenesis of cardiometabolic diseases. Recently however, the nontraditional effects of protease have been elucidated. Proteases have been shown to regulate signaling cascades such as the Akt and mTOR signaling pathways. These novel insights not only add to our growing understanding of the role of proteases in cardiometabolic diseases but would also provide novel avenues of treatment of this disease (Fig. 1). More studies are warranted to understand the cross-talk between proteases and cellular signaling pathways. Such studies, together with already published work would provide a strong rationale for clinical trials which would allow the harnessing of protease inhibitors in treating cardiometabolic diseases.

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Highlights

- Proteases play a pivotal role in the pathophysiology of cardiometabolic diseases
- Matrix metalloproteases, calpains, cathepsins and caspases are among the proteases that have been studied extensively
- This review focuses on the functional and mechanistic studies of proteases in the context of cariometabolic disease
- Proteases represent a potential target for treatment of cardiometabolic disease

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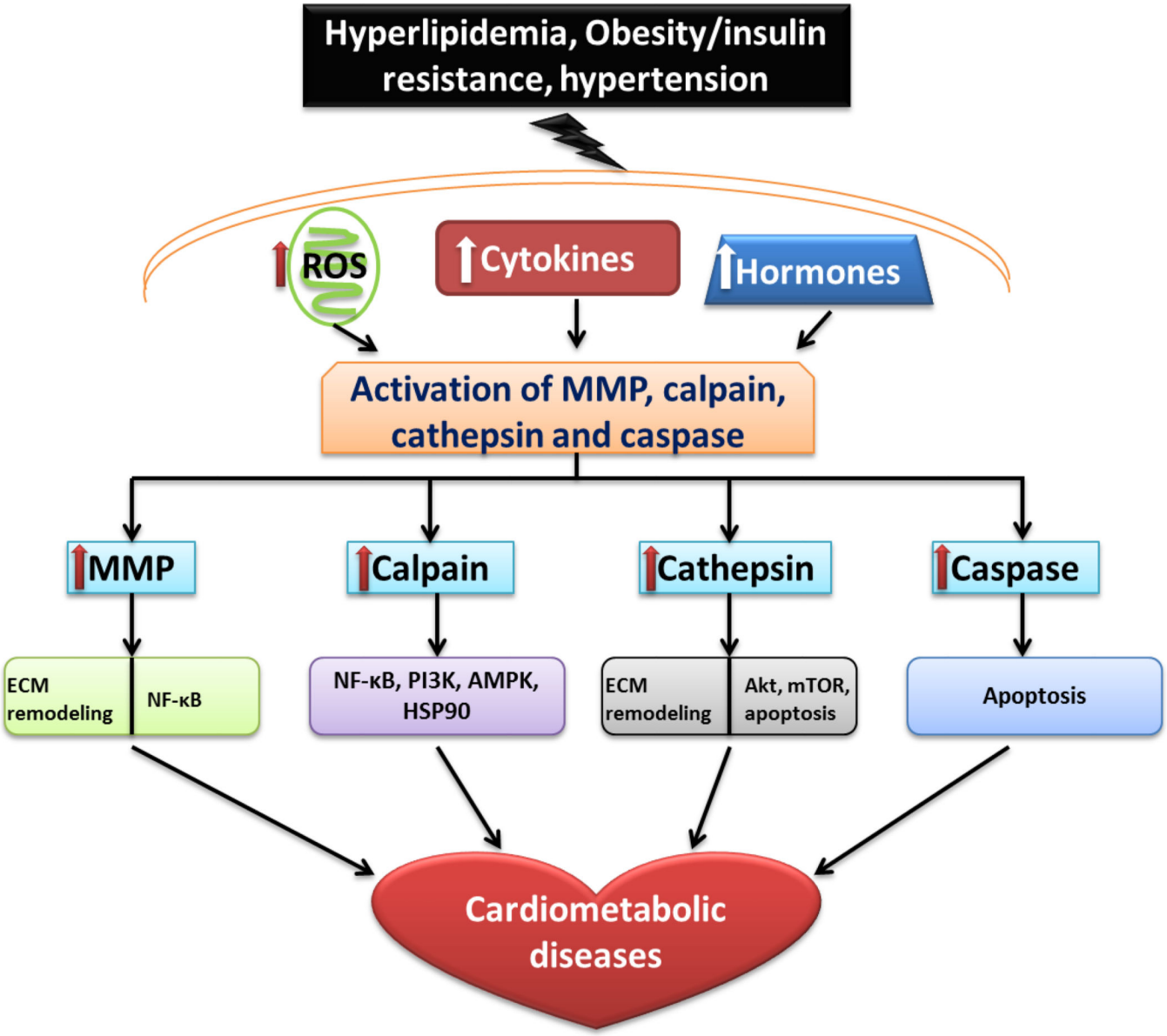

Figure 1.

Role of proteases in cardiometabolic disease. Proteases (MMP, calpain, cathepsin and caspase) affect ECM remodeling and participate in a variety of intracellular signaling pathways in response to elevated ROS, cytokines and hormones contributing to the pathogenesis of cardiometabolic diseases. MMP: matrix metalloproteinase. ECM: extracellular matrix. NF-κB: nuclear factor-κB. PI3K: phosphoinositide 3-kinase. AMPK: AMP-activated protein kinase. HSP90: heat shock protein 90. mTOR: mammalian target of rapamycin.