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Review

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 countries. Metabolic syndrome, including obesity, diabetes/insulin resistance, hypertension and dyslipidemia is a 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 over-express 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 treating or controlling these diseases. This article is part of a Special Issue entitled: Autophagy and protein quality control in cardiometabolic diseases.

1. Introduction

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/

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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 proteases including matrix metalloproteinase (MMP), calpain, cathepsin and caspase are among the 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 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 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 structures, 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 MMP 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 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 are ill-defined, it is widely accepted that amino acid sequence rich in proline, glutamic acid, serine and threonine elevates 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 activities. 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