Cysteine Protease Cathepsins in Atherosclerosis-Based Vascular Disease and Its Complications

Xian Wu Cheng, Zhe Huang, Masafumi Kuzuya, Kenji Okumura, Toyoaki Murohara

Abstract—Atherosclerosis-based vascular disease is an inflammatory disease characterized by extensive remodeling of the extracellular matrix architecture of the arterial wall. Although matrix metalloproteinases and serine proteases participate in these pathological events, the discovery of cysteine protease cathepsins, such as cathepsins K, S, L, and B, and cystatin C, and their tissue distribution has suggested that at least some of them participate in cardiovascular disease. Studies on vascular cells have shown that atherosclerosis-associated inflammatory cytokines augment cysteinyi cathepsin expression and activity. Novel insight into cathepsin functions has been made possible by the generation and in-depth analysis of knockout and transgenic mice. These studies have provided direct evidence implicating cathepsins in atherosclerosis-based vascular disease through the activation, liberation, and modification of angiogenic growth factors, cytokines, and proteases associated with lipid metabolism, cell events (migration, invasion, proliferation, and apoptosis), angiogenesis, and matrix protein remodeling. Furthermore, evaluation of the feasibility of cathepsins as a diagnostic tool has revealed that the serum cathepsins S and L and the endogenous inhibitor cystatin C hold promise as biomarkers of coronary artery disease and aneurysm formation. The goal of this review is to summarize the available information regarding the mechanistic contributions of cathepsins in atherosclerosis-based vascular disease. (Hypertension. 2011;58:00-00.)

Key Words: cysteinyi cathepsins ■ cystatin C ■ extracellular matrix degradation ■ cell events ■ vascular disease

Extracellular matrix proteins (ECMs), such as elastin and collagens, contribute to the structural integrity of the cardiovascular wall. ECM remodeling is one of the underlying mechanisms in atherosclerosis-based vascular disease (AVD). Extracellular proteases, such as matrix metalloproteinase (MMP) and serine protease families, can degrade the ECM and thereby contribute to cardiovascular pathogenesis. Genetic and pharmacological interventions using MMPs and serine proteases have been shown to result in incomplete suppression of cardiovascular and renal remodeling in animal models, suggesting other proteases, such as cysteine proteases, also contribute to AVD.

Cathepsins of the cysteine protease family were originally identified as proteases acting in the lysosome. The previous studies presented focused on the cysteinyi cathepsin family, which is composed of 11 human members to date (cathepsins B, C, H, F, K, L, O, S, V, W, and X/Z), and will be collectively abbreviated hereafter simply as cathepsins. These cathepsins are primarily intracellular proteases that function in terminal protein degradation in lysosomes and protein processing in other intracellular organelles, such as hormone secretory granules. Recently, cathepsins have also been demonstrated to play an important role in ECM remodeling and are implicated in the development and progression of AVD. Furthermore, evaluation of the feasibility of cathepsins as a diagnostic tool has revealed that serum levels of several cathepsins seem to be promising biomarkers in the diagnosis of ischemic heart disease and atherosclerotic plaque complications. This review examines several issues concerning the biological roles and molecular functions of cysteine proteases in vascular pathological processes, especially with respect to their potential application as diagnostic and/or prognostic markers and drug targets.

Cathespin Molecular Regulation

Cathepsin Regulation

Many studies have been done to analyze the promoter regions of the human cathepsin genes, as well as to understand the regulation of different splice variants. It has been reported that there were alternative promoters and alternative splicing for cathepsins B and L in tumor cells. An article by Mohamed and Sloane reviews cathepsin transcript variants in tumor tissues and tumor-related cells comprehensively. In addition, Ets family transcription factors have been linked to the transcriptional regulation of cysteine proteases. In macrophages, Ets family transcription factors regulate the...
expression of cathepsin K\textsuperscript{19} and cathepsin C.\textsuperscript{20} Therefore, transcriptional regulation by Ets family members might contribute significantly to the increased expression of cathepsins in human disease. However, we still know little about the transcriptional and translational regulation of cathepsins in AVD and AVD-associated cells. In addition, the major regulators of cathepsin activity are their endogenous inhibitor cystatin family (reviewed in Reference\textsuperscript{21}).

**Cathepsin Biosynthesis, Processing Activation, and Trafficking**

Similar to other proteases, the cathepsins are synthesized as inactive proenzymes and are activated by proteolytic removal of the N-terminal propeptide. The immature protein possesses an N-terminal proregion, which is removed to activate the enzyme, suggesting that the proregion acts as an autoinhibitor (reviewed in Reference\textsuperscript{22}). Cathepsins are further processed in the Golgi apparatus by modification of mannose residues. After acidification in the late endosomes, cathepsins become active and begin proteolytic processing, with cleavage within the proregion allowing proregion dissociation from the enzyme. Active cathepsins have been recruited from late endosomes or lysosomes for secretion into the extracellular space via Ca\textsuperscript{2+}-mediated fusion of these organelles with the plasma membrane.\textsuperscript{23} In addition, some cathepsins do not target to endosomes or lysosomes for maturation but rather secreted into the extracellular milieu as proenzymes (called exocytosis).\textsuperscript{24} The cathepsin maturation has been covered well by a recent review.\textsuperscript{22}

**Cathepsin Activity**

CysteinyI cathepsins are localized on cell membranes and secreted and localized in endosomal or lysosomal vesicles, suggesting that their enzymatic substrates and functions might change along with their localization. Cavallo-Medved and Sloan\textsuperscript{e24} proposed that the association of cathepsins with caveolae in endothelial cells (ECs) is associated with their ability to degrade extracellular matrices. One mechanism of cathepsin B association with the cell surface is through an interaction with annexin II heterotrimers. Annexin II induces cathepsin B to caveolae,\textsuperscript{26} plasma membrane surfaces with high proteolytic capacity harboring a wide variety of interdependent proteases, for example, membrane-bound MMPs and serine proteases of the plasminogen activator/plasminogen system.\textsuperscript{28} Recently, we demonstrated that active cathepsin S was colocalized with integrin \(\alpha\beta3\) as a receptor on the vascular smooth muscle cell (SMC) surface, playing an important role in SMC-mediated ECM degradation.\textsuperscript{27} Once they are localized on cell surfaces, cathepsins may act on the contact sites of cardiovascular cells and AVD-associated cells with the basement membrane. These areas may be often acidified by those cells, thereby generating conditions that are favorable for activation of secreted inactive cathepsinzymogens to active forms. Active cathepsins S, K, and L have been shown to be able to degrade the protein components of basement membranes and the interstitial connective matrix, including laminin,\textsuperscript{28} fibronectin,\textsuperscript{28,29} elastin,\textsuperscript{15,30} and collagen.\textsuperscript{27,28,31,32} It has been reported the use of bafilomycin, an inhibitor of acidification of both extracellular and intracellular spaces, that it inhibited macrophage elastase activity and that these extracellular acidic milieux resulted from increased expression of vacuole-type H+-ATPase (reviewed in Reference\textsuperscript{15}). This finding provides a reasonable explanation for ECM degradation by cysteine cathepsins released from vascular cells and inflammatory cells (reviewed in Reference\textsuperscript{23}). Novel insight into cathepsin function was made possible by the generation and in-depth analysis of knockout and transgenic mice.\textsuperscript{34} These studies established that cathepsins are critically involved in the proteolytic processing of specific substrates in AVD processes.

**Cathepsin Function: Mechanisms of Action on Molecular and Cellular Levels**

**Cathepsins and Immune and Inflammatory Actions:**

**Antigen Presentation and Response to Inflammatory Cytokines**

In addition to their role in ECM degradation, cathepsins are involved in the immune action of presentation of major histocompatibility complex (MHC) class II antigen. MHC class II \(\alpha\beta\) heterodimers assemble in the endoplasmic reticulum with the assistance of the invariant chain (Ii; reviewed in Reference\textsuperscript{35}). Cathepsin S has been described to degrade MHC class II-associated Ii in cardiovascular disease–associated professional antigen-presenting cells, such as macrophages and dendritic cells.\textsuperscript{36–39} Previous study demonstrated that dendritic cells developmentally regulate the transport and surface expression of MHC class II molecules to control their capacity for antigen presentation by the ratio of cystatin C:cathepsin S.\textsuperscript{39} Interestingly, deficiency of both cathepsin S and L could process Ii and load peptide onto MHC class II dimers normally in macrophages, whereas both processes were blocked by cathepsin inhibitors.\textsuperscript{34} Cathepsin S deficiency impairs antigen presentation by bone marrow–derived antigen-presenting cells but does not affect CD4\textsuperscript{+} T-cell development.\textsuperscript{40} Cathepsin L has been shown to affect the immune response by regulating the levels of \(\alpha 5\), \(\alpha 6\), and \(\beta 1\) integrin chains in T cells. Recently, Sun et al\textsuperscript{41} demonstrated that invariant chain deficiency lessened the release of antigen-presenting cell-derived T-helper 1/T-helper 2 cytokines and CD25\textsuperscript{+} activated T cells and reduced atherosclerotic lesion formation in low-density lipoprotein receptor-deficient (Ldlr\textsuperscript{−/−}) mice. On the other hand, inflammatory macrophages and leukocytes account for most of the cysteine cathepsin expression in human and animal atherosclerotic arteries and/or failing myocardium and kidney.\textsuperscript{9,11,13,42–44} In vitro studies from our group and those of other researchers have demonstrated that, although monocytes, macrophages, and/or T cells express negligible levels of cathepsins S and L under basal conditions, these cells are sensitive to neurohormone (angiotensin II) and/or inflammatory cytokines (tumor necrosis factor-\(\alpha\) and interleukin 1\(\beta\)) for the expressions and secretions of their cathepsins.\textsuperscript{42–45} The authors of a previous study reported that deficiency of cathepsins S or L reduced inflammatory actions and diet-induced atherogenesis in Ldlr\textsuperscript{−/−} mice.\textsuperscript{13,48} These findings raise the possibility that the cardiovasculoprotective effects mediated by cathepsins S or L deficiency are likely attributable, in part, to the attenuations of immune and inflammatory actions.
Cathepsins and Cell Events

Adhesion and Migration
In the progression of inflammatory diseases, such as atherosclerosis, the first step may involve leukocyte recruitment from the circulation by adhesion to the endothelium.46 Current understanding implicates specific adhesion molecules expressed on the surface of vascular ECs, for example, vascular cell adhesion molecule 1, and chemoattractant molecules, such as macrophage chemoattractant protein 1, in this process.46 Cathepsin S deficiency reduced the levels of circulating these molecules in a mouse model of diet-induced atherosclerosis.13 Therefore, cathepsin S may act like MMPs and release adhesion molecules from the surface of ECs.47 Alternatively, cathepsin S may indirectly influence the production of adhesion molecules by affecting the γδ-T lymphocyte population.48 In addition, cell invasion, such as transmigration of adhered cells or media SMCs through the basement membrane or internal elastic lamina, is one of the most important steps contributing to atherosclerotic lesion and neointimal formation. There is increasing evidence that cathepsins promote cell invasion by ECM remodeling in the cardiovascular wall. SMCs have been shown to contain immunoreactive cathepsins S, K, and L in the affected vessel walls of atherosclerotic humans and animals, notably near sites of internal elastic lamina fragmentation,5,13 which can break down the elastic barriers. Several lines of evidence support this hypothesis. We recently demonstrated in vitro that chemoattractant protein-mediated SMC invasion was sensitive to the broad spectrum inhibitor of cathepsins trans(epoxy)succinyl-L-leucylamido-(4-guanidino)butane (E64) or the cathepsin S selective inhibitor morpholinurea leucine-homophenylalanine-vinylsulfone-phenyl (LHVS).27 Furthermore, leucine-homophenylalanine-vinylsulfone-phenyl also significantly inhibited monocyte transmigration through the collagen matrix gel into the SMC layer.5 Cathepsin S-null monocytes yielded similar results,27 suggesting the involvement of cathepsin S and possibly other cysteine proteases in monocyte and SMC transmigration.

Proliferation
Recently, cysteiny1 cathepsins were also discovered to influence the regulation of cell proliferation by cell signaling. Inhibition of cathepsins by a synthetic broad-spectrum cysteine protease inhibitor has been found to significantly reduce tumor cell proliferation in a mouse model of pancreatic islet tumor cells.49 Deficiency of cathepsins B or L decreased tumor cell proliferation and tumor growth.50 Cathepsin X has been shown to suppress proliferation of mononuclear cells by activation of MAC-1 (CD11b/CD18), but it was also shown to increase the proliferation of T lymphocytes by activation of LFA-1 (CD11a/CD18).51 Cathepsin B also suppresses proliferation of peripheral blood mononuclear cells.52 A single recent study has shown that the reduction of Cux1 processing by cathepsin L deletion results in the accumulation of Cux1, downregulation of p21/p27, and increased cell proliferation.53 On the other hand, cystatin C deficiency resulted in an increased Ki67 proliferation index and epidermal hyperplasia, most likely attributed to enhanced activity of cysteine cathepsins and loss of cystatin C activity in antagonizing cell proliferation in K14-HPV16 transgenic mice.54 However, the effects of cathepsins on cell proliferation seem to be quite cell dependent, because several groups, including our own, have reported that genetic and pharmacological inhibition of cathepsin S exhibited effects on neither platelet-derived growth factor-BB–induced SMC proliferation nor vascular endothelial growth factor–induced EC proliferation.55

Apoptosis
Apoptosis promotes the development of AVD. Although the importance of caspases in the apoptotic process is firmly established, the role of the lysosomes and lysosomal enzymes in cell death has been unmasked only recently by several means. Strikingly, the antiapoptotic molecules Bcl-2, Bcl-xL, Mcl-1, and X chromosome-linked inhibitor of apoptosis are targeted by the lysosomal cathepsins B, L, K, and H in several human cancer cell lines,56 suggesting that cathepsins can mediate caspase-dependent apoptosis downstream of the mitochondria. Data from our and that of those of collaborators showed that suppression of cathepsin activity by L-3-transcarboxylycysteine (E64d) inhibits Q27-induced cardiomyocyte apoptosis,11 and cystatin C deficiency enhances epithelial apoptosis.54 It is well known that massive lysosomal rupture can induce necrotic autolysis of cells, a process that is mediated by the lysosomal cathepsins and other “acidic” hydrolases. In vitro studies using cultured macrophages have shown that both 7β-hydroxycholesterol and oxidized low-density lipoprotein induced lysosomal destabilization, leading to leakage of cathepsins B and L to the cytoplasm, activation of caspases, and subsequent apoptosis.57 Thus, this proapoptotic mechanism may also apply to other cells in the progression of cardiovascular disease, such as SMCs and ECs.

Cathepsins and Lipid Metabolism

Lipid Uptake and Modification
Lipoprotein modification and uptake by atherosclerotic lesion cells, namely macrophages and SMCs, are important pathological steps in atherosclerotic lesion formation.33,35,58–60 Several cathepsins have been implicated in apolipoprotein B-100 proteolytic modification, which enhances extracellular low-density lipoprotein particle aggregation, lipid droplet formation, and low-density lipoprotein retention to arterial proteoglycans.60 After taking up the lipoproteins and modified lipoproteins, macrophages and SMCs become foam cells filled with lipid droplets. In addition, lipids or modified lipoproteins also affect cysteiny1 cathepsin cellular expression and localization. Sun et al61 demonstrated that free cholesterol accumulation stimulated cathepsin K expression via activation of toll-like receptors and p38 mitogen-activated protein kinase. When macrophages were exposed to oxidized low-density lipoprotein or 7β-hydroxycholesterol, these cells expressed high levels of cathepsins B and L, in addition to forming foam cells.57 These cathepsins, which translocated from lysosomes to cytosol or nuclei, cause foam cell apoptosis in the development and progression of atheroma.

Cholesterol Efflux
The literature provides ample evidence that cathepsins are also involved in cholesterol efflux. Cysteiny1 cathepsins
participate in the degradation of high-density lipoproteins, thus reducing macrophage foam cell cholesterol efflux.62 In vitro, recombinant human cathepsins S and F degraded apolipoprotein A, leading to complete loss of apolipoprotein A-I cholesterol acceptor function, thereby blocking macrophage intracellular cholesterol efflux.63 In contrast, under the same conditions, recombinant human cathepsin K did not act similarly.64 Therefore, different cathepsins show different functions in lipid metabolism and contribute to atherosclerosis via different mechanisms. In general, then, the role of cathepsin cysteine proteases in lipid uptake, storage, and efflux has been partly elucidated (reviewed in References35 and62). However, the most important question of whether the role of cathepsins in lipid metabolism is atherosclerosis stimulating or protective remains unclear.

Cathepsins in AVD and Its Complications

Atherosclerotic Lesions and Restenosis

Atherosclerotic lesions contain much higher levels of cathepsin S and K mRNAs and proteins than do normal arteries.9 Early lesions show cathepsin K expression in the intimal and medial SMCs, whereas, in advanced atherosclerotic plaques, cathepsin K is localized mainly in macrophages and SMCs of the fibrous cap.9 In addition to atherosclerotic lesions, increased expression of cathepsins S and K has been reported in the neointima of arteries in a balloon injury model of restenosis.12,28 In vitro experiments revealed that, although these vascular cells and macrophages express negligible levels of cathepsins S, K, and L, incubation of these cells with inflammatory cytokines, such as interleukin 1β, tumor necrosis factor-α, and interferon γ, significantly induces the expression and secretion of those cathepsins and their collagenolytic and elastolytic activities.15,27 This suggests that inflammatory processes that prevail during atherosclerotic lesion and neointima formation locally increase the activities of these cathepsins. The ability of SMCs and macrophages to use cathepsins to degrade elastin and collagen supports the idea that these proteases play a role in vessel wall remodeling in humans and animals. This notion is further supported by the direct evidence that genetic deletions of cathepsins and ApoE double-deficient mice display reduced expression of cathepsin K-null mice showed no difference in AAA formation between both genotypes mice. Therefore, although cathepsins can contribute to AAA with different mechanisms, the contradictory observations between the Bai and Sun groups could be attributed to the difference experimental models. Based on the observations from both laboratory investigations,70,72 Sun et al70 raise the possibility that angiotensin II infusion enhances both peripheral active CD44/CD255 T cells and Lgg6G leukocytes and AAA lesional CD45+ leukocytes and mac-3+ macrophages, and thereby both hyperinflammatory responses may have diminished the cathepsin K deficiency-mediated vascular protective actions.71

Atherosclerosis-Related Vasa Vasorum

Recent observations indicate that atherosclerosis-related vasa vasorum can cause plaque growth and instability and rupture of advanced atherosclerotic lesions in human aortas.73 Accumulating evidence shows that MMPs are implicated in hypoxia-induced neovascularization,74,75 and MMP inhibition impaired plaque neovessel formation and growth.76 Recently, the roles of cathepsins in angiogenesis have been tested using in vivo and in vitro experimental models. Our data and those of colleagues demonstrated that deficiency of cathepsin S impaired microvessel growth despite normal levels of the angiogenic factors basic fibroblast growth factor and vascular endothelial growth factor.77 Cathepsin S controls angiogenesis and tumor growth via matrix-derived angiogenic factors, such as laminin S-derived proangiogenic-γ2 and type collagen-derived antiangiogenic peptides.77 Recently, a gene chip expression assay and bone marrow transplantation assay demonstrated that cathepsin L was more highly expressed in EPCs than in mature ECs and that this cathepsin plays a crucial role in the homing of EPCs to the ischemic vasculature.78 Until now, in vivo intervention studies using inhibitors or genetically modified mice to define the role of cathepsins in atherosclerosis-related vasa vasorum have been lacking (Table).

Aneurysm Formation

Abdominal aortic aneurysm (AAA) formation is characterized by extensive medial and adventitial inflammatory cell invasion, medial SMC depletion, and degradation of elastin and collagen in the media.65 As in atherosclerotic lesions, these inflammatory infiltrates release cathepsins and cause further destructive elastinolysis, inflammatory cell recruitment, vascular cell apoptosis, and angiogenesis.65,66 In human AAA, protein levels of cathepsins K, L, and S are increased, whereas the level of their endogenous inhibitor, cystatin C, is decreased.15,67 Similarly, cathepsins B, K, and S are highly expressed in cerebral aneurysms, whereas cystatin C is sparse.68 Mice deficient for cystatin C developed increased lumen diameter and AAA lesion size in response to angiotensin II.69 These mice also showed enhanced inflammatory cell accumulation, more severe elastin break, and fewer SMCs in the tunica media than the control mice (Table). Recently, Sun et al70,71 have demonstrated that cathepsin L or K reduces elastase perfusion-induced AAAs in ApoE−/− mice. These findings suggest that imbalanced proteolytic activities in the vascular wall, resulting in excessive matrix destruction and progressive weakening of the arterial wall, are among the hallmarks of AAA pathology. It should be noted that Bai et al72 have reported that cathepsin K-null mice showed no difference in AAA formation between both genotypes mice. Therefore, although cathepsins can contribute to AAA with different mechanisms, the contradictory observations between the Bai and Sun groups could be attributed to the difference experimental models. Based on the observations from both laboratory investigations,70,72 Sun et al70 raise the possibility that angiotensin II infusion enhances both peripheral active CD44/CD255 T cells and Lgg6G leukocytes and AAA lesional CD45+ leukocytes and mac-3+ macrophages, and thereby both hyperinflammatory responses may have diminished the cathepsin K deficiency-mediated vascular protective actions.71

Plaque Rupture, Thrombosis, and Calcification

Mechanistic studies of plaque rupture have been difficult because of a lack of reliable animal models; the current available model developed by our group uses the carotid arteries of ApoE−/− mice that received ligation and polyethylene cuff replacement approximately.79 Features of fibrous cap ruptures and buried fibrous caps in animals have been used to evaluate lesion stability,80 but whether these structures are comparable with human plaque ruptures is still debatable. This model has been used to study the role of cathepsin S in lesion stability. Cathepsin S and ApoE double-deficient mice display fewer plaque ruptures and buried
fibrous caps than control mice (Cheng et al, unpublished data, 2011). Cathepsin K deficiency induces diet-induced atherosclerotic plaque fibrosis in ApoE−/− mice. On the other hand, thrombotic complications of atherosclerosis often involve plaque rupture and cause most of the acute manifestations of atherosclerosis, such as myocardial infarction or stroke. Indeed, thrombus formation usually results from physical disruption of atherosclerotic plaque and appears to be related to the level of collagen in the lesion’s fibrous cap. There has been only a single study examining the role of cysteine proteases in thrombosis during atherogenic complication. In that study, cathepsin S deficiency displayed a prothrombotic phenotype. After photochemical carotid artery injury, the time to the development of occlusive thrombosis decreased in cathepsin S–null mice. The accelerated thrombotic response to arterial injury and the shortening of plasma clotting times accompanied an increased activity of coagulation factors and plasma von Willebrand factor in cathepsin S–deficient mice. These results suggest that cathepsin S exhibits antithrombotic properties. However, the mechanism by which cathepsin S affects thrombosis and whether its antithrombotic properties impact atherosclerosis remain undetermined.

**Table.** Cathepsins (Cats) S, K, L, and Cystatin (Cyst) C in Atherosclerosis-Based Disease, Its Complications, Therapies, and Biomarkers (Expression and Genetic Phenotypes)

<table>
<thead>
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<th>Diseases and Implications</th>
<th>ECM In Vivo</th>
<th>Cat S</th>
<th>Cat K</th>
<th>Cat L</th>
<th>Cyst C</th>
<th>Reference</th>
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<td>Expression</td>
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Cat−/− indicates cathepsin deﬁciency; cyst C−/−, cyst C deﬁciency; Apo−/−, apolipoprotein E deﬁciency; Ldlr−/−, low-density lipoprotein receptor deﬁciency; NS, no signiﬁcant change; coll, collagen. (+) indicates increase; (−) indicates decrease; − indicates no study; ARB, angiotensin receptor blocker; SMC, smooth muscle cell; AAA, abdominal aortic aneurysm; ECM, extracellular matrix protein.

*Data are from Cheng et al, unpublished data, 2011.*
In addition, cardiovascular calcification commonly causes devastating complications, including plaque rupture\(^82–^84\) and aortic valve stenosis,\(^85\) which currently have no suitable therapeutic alternative beyond valve replacement. Previous studies suggest that arterial calcification occurs through highly regulated molecular processes characterized by the expression of osteogenic proteins and matrix-degrading proteinases. During atherogenesis, macrophage-derived elastases, such as elastolytic cathepsins or MMPs (MMP-2 and MMP-9), have been shown to degrade medial elastin, which favors calcification through an increase of elastin polarity that, in turn, enhances elastin affinity for calcium (Sasaki et al, unpublished data, 2011).\(^86,^87\) Recently, the data from cathepsin S–deficient mice have provided new insights into the pathobiology of arterial calcification and have aided the investigation of novel therapeutic strategies to reduce the onset of cardiovascular events and, thus, mortality (Table).\(^88\)

**Pharmacological Therapeutics for Cathepsins in AVD**

**Targeting Cathepsins as Part of a Proteolytic Pathway**

Over the past decade, several pharmaceutical companies have become interested in cysteine protease inhibitor development. Individual cathepsins involve physiological and pathophysiological processes, although redundancies may exist, favoring the application of pharmacological inactivation of each individual cathepsin using its selective inhibitors. For the development of selective cathepsin inhibitors, it has mainly focused on cathepsins S and K because of their involvement in osteoporosis\(^32\) and cancer.\(^16,^49\) To our knowledge, compound 6, a nitrile-based specific cathepsin S inhibitor was applied for the first time to evaluate cathepsin inhibitor-mediated vasculoprotective effects on atherosclerosis in an experimental animal model. Cathepsin S inhibitor treatment mice display fewer elastic lamina breaks, infiltrated macrophages, and buried fibrous caps and lessen atherosclerotic plaque size in \(\text{ApoE}^{-/-}\) mice.\(^89\) Recently, we demonstrated that, in vivo, administration of a broad-spectrum synthetic cathepsin inhibitor E64 lessened hypertension-induced cardiac and renal fibrosis and dysfunction in a Dahl salt-sensitive rat model.\(^11,^43\) However, there is limited available information regarding these cysteine protease inhibitors in treating cardiovascular diseases.

**Cardiovascular Drug-Mediated Cathepsin Expression Reduction**

The diversity in the expression of cathepsins and their endogenous inhibitors in the cardiovascular-renal system and AVD-associated cells suggests that individual enzymes have distinct roles in AVD progression and that these roles depend on the disease origin. Angiotensin inhibition decreased the progression of advanced atherosclerosis and improved plaque instability by inhibition of the mRNA and activity/protein of the elastolytic proteases cathepsin S and MMP-9.\(^90\) A study in humans indicated that statins improved cathepsin S and cystatin C balance, which has been implicated in aneurysm formation.\(^91\) Cysteine cathepsins were discovered recently to be targeted by several cardiovascular field drugs (Table) by several proposed mechanisms. Strikingly, simvastatin can inhibited cardiac hypertrophy and fibrosis in \(\text{ApoE}^{-/-}\) mice fed a high-fat diet via a reduction of cathepsin S and MMP-9 expressions in association with increasing peroxisome proliferator-activated receptor-\(\alpha\) and -\(\gamma\) expressions.\(^92\) Systemic administration
of ribbon-type decay oligodeoxynucleotide prevented AAA by inhibiting the secretion of the nuclear factor κB–mediated cathepsins B and K in macrophages.93

Circulating Cathepsins as Biomarkers for AVD
Enhanced expression of the cathepsins S and K in human AAA lesions with concomitant deficiency of their endogenous inhibitor, cystatin C, have been reported.9,67 Serum cystatin C levels are significantly lower in AAA patients than in control subjects.82,74,75 The expressions and activities of cathepsins B, C, and L also were increased in the aneurysm wall and thrombus of human aortic aneurysms when compared with normal arteries (Table).15,95,96 In addition to AAA, serum cathepsins S and/or L were increased in patients with coronary artery ectasia and atherosclerotic stenosis,14,97,98 suggesting that these cysteiny1 cathepsins may participate in coronary artery restenosis and aneurysm formation.

Clinical Implications
Recent studies have uncovered multiple divergent roles for different cathepsins in AVD (including atherosclerosis, aneurysm, and vasa vasorum) and its complications. From the findings in the different organs and different cells (cardiovascular cells and AVD-associated cells) discussed above, it has become clear that cysteiny1 cathepsins serve as regulatory enzymes beyond acting as simple housekeeping proteases and harbor important functions outside the lysosome. Novel insights into cathepsin function have been made possible by the generation and in-depth analysis of knockout and transgenic mice. These studies have provided direct evidence that cathepsins are implicated in AVD through their activation, liberation, and modification of angiogenic growth factors, cytokines, and proteases associated with degradation of lipid metabolism, cell events (migration, invasion, proliferation, and apoptosis), angiogenesis, and matrix protein remodeling (Figure). The current quest for cathepsins as a diagnostic tool, therefore, seems a reasonable goal in cardiovascular disease research. Furthermore, cathepsins have been targeted by pharmacological drugs and inhibitors. However, until now no data were available on the effect of these inhibitors in AVD.

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Disclosures
None.

References


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