Role for Cysteine Protease Cathepsins in Heart Disease
Focus on Biology and Mechanisms With Clinical Implication

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The extracellular matrix (ECM) of the heart is composed largely of elastin and collagen and plays many roles in cardiac wall and valve homeostasis. Maintenance of a healthy cardiac system relies on controlled biosynthesis, maturation, function, and breakdown of ECM proteins. Dysregulation of proteolytic enzymes may disrupt these normal biological processes in myocardium-coronary-valve disease (CCVD).

Substantial evidence supports the involvement of matrix metalloproteinase (MMP) and serine protease families in this process (reviewed elsewhere). Cysteinyl proteases have received much less consideration in this regard, even though cardiovascular cells and macrophages with greatly expanded lysosomal compartments figure prominently in the pathogenesis of CCVD.

Lysosomal cysteine proteases, generally known as cathepsins, were discovered in the second half of the 20th century. In the initial years after their discovery, cysteine cathepsins were shown to localize in lysosomes and endosomes and to function there to degrade unwanted intracellular or endocytosed proteins. However, the recent recognition of the inducible cathepsins F, K, S, B, and L led to the unraveling of their molecular functions in inflammatory and/or autoimmune diseases such as atherosclerosis, obesity, rheumatoid arthritis, cardiac repair, cardiomypathy, and cancer. Most strikingly, we have now discovered that these cathepsins can be secreted into and function within the extracellular spaces. The observations of cathepsin expression and activity in failing cardiac tissues and valve tissues from humans and animals and in cultured media of cardiomyocytes, cardiac fibroblasts, vascular smooth muscle cells, endothelial cells, and macrophages have significantly broadened our understanding of their potential roles in cardiovascular pathogenesis. Furthermore, recent studies have shown that pharmacological cathepsin inhibition exhibits cardiovascular protective actions in animal models. In addition, accumulating evidence shows a prognostic and diagnostic impact of circulating and tissue cathepsins and/or the endogenous inhibitors known as cystatins in cardiovascular injury, remodeling, and function. This review focuses on recent findings in this field, highlighting the cathepsin biology and the significance of lysosomal cysteine proteases in ECM remodeling, pharmacological intervention, and prediction of the development and progression of heart disease.

Biology of Cysteiny Cathepsins

The Properties and General Structure of Cysteinyl Cathepsins

Modern molecular biology has permitted the identification of various characterization of cysteine protease cathepsins, including the following: (1) Cathepsins have a high homology with members of the papain family; (2) they comprise mainly endopeptidases with a few exceptions (cathepsin B, exopeptidase; cathepsin H, aminopeptidase); (3) they are synthesized as glycosylated precursors that are activated by removal of the N-terminal propeptide by other proteinases or autocatalysis; (4) they have a broad substrate specificity for cell matrix components and can degrade almost all intracellular and extracellular proteins through their combined activities (Table 1); (5) their activity is regulated intracellularly by a specific endogenous inhibitor cystatin subgroup called stefins (stefin A and B) and extracellularly by cystatins (cystatin C) and kininogens; and (6) they have cell- or tissue type-specific (as in cathepsins F, S, and K) or nonspecific (cathepsins B, H, and L) expression patterns. In humans, cathepsin cysteine proteases consist of a family of 11 members (cathepsins B, C, F, H, K, L, O, S, V, W, and Z) in mice, 19 cathepsins have been discovered, including several additional placently expressed cathepsins with no human homolog. As described in a previous review, most of these cathepsins are relatively small proteins with $M_r$ values in the range of 20 000 to 35 000. Human cathepsins have been shown to share a conserved active 3-dimensional pocket formed with histidine, aspartagine, and cysteine residues. At its N-terminus structure, cathepsin contains a small minidomain, which forms a small compact structure, and an extended peptide, which is bound to the cysteine residue at the C-terminus.
over the active site cleft, occluding it. As shown in models of the primary cathepsin structure, the cathepsin contains a signaling peptide, proregion, heavy chain, and light chain (Figure 1A). The D-dimensional structures of cysteinyl cathepsins show that all enzymes share a common fold. Analysis of the crystal structure of cathepsin L shows that the mature enzymes consist of 2 domains separated by a V-shaped active site cleft where Cys25 and His163 form the catalytic site. In contrast, aminopeptidase cathepsin H has been shown to use the minichain, an 8-residue-long peptide originating from the propeptide region, which binds into the nonprimed region of the active site cleft in a substrate orientation. These findings suggested that the binding sites within the active site cleft provide the structural basis for understanding the enzyme specificity. This notion is further supported by the observations that the crystal structure of cathepsins in complex with the major histocompatibility class II-p41 Ii fragment provides the basis for differentiation between the structurally highly similar cathepsins S and L, thus contributing to a better understanding of their roles in cellular processes.

Table 1. Cathepsin Expression in Cardiovascular and Other Cells: Its Regulation, Substrates, and Events

<table>
<thead>
<tr>
<th>Cathepsins</th>
<th>Gene Expression Stimulatory Factors</th>
<th>Substrates</th>
<th>ECM Degradation Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsin B</td>
<td>Ang II, IL-1β, TNF-α, IFN-γ</td>
<td>Plasminogen, antiapoptotic molecules (Bcl-2, Bcl-xL, Mcl-1, and XLAP)</td>
<td>-/−</td>
</tr>
<tr>
<td>Cathepsin S</td>
<td>Ang II, IL-1β, TNF-α, IFN-γ, VEGF, bFGF, H₂O₂</td>
<td>Elastin, collagen, fibrinectin, laminin, MHC class II Ii</td>
<td>+/+</td>
</tr>
<tr>
<td>Cathepsin K</td>
<td>Ang II, IL-1β, TNF-α, IFN-γ</td>
<td>Elastin, collagen</td>
<td>+/+</td>
</tr>
<tr>
<td>Cathepsin L</td>
<td>IL-1β, TNF-α, VEGF, bFGF, glucose</td>
<td>Prohormones, MHC class II Ii, trypsinogen</td>
<td>+/+</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>Ang II, IL-1β, TNF-α, IFN-γ, H₂O₂</td>
<td></td>
<td>+/+</td>
</tr>
</tbody>
</table>

EMC indicates extracellular matrix; Ang II, angiotensin II; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; XLAP, X-chromosome-linked inhibitor of apoptosis; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; MHC, major histocompatibility class; +, genetic mice report or genetic mice–derived cell assay; and −, no genetic mice report.

Figure 1. A model of the cathepsin primary structure and cathepsin maturation processes. A, Cathepsin contains a signaling peptide, proregion, heavy chain, and light chain. B, The cathepsin maturation process is as follows: synthesis→targeting to endoplasmic reticulum (ER)→signal peptide removing/folding and disulfide-bound formation/glycosylation (ER)→mannose-6 residue phosphorylation (m6p; Golgi)→acidification (early endosome)→proregion removing (endosome)→further activation (heavy and light chains, endosome)→Ca²⁺-mediated organelle fusion and secretion into extracellular spaces. It should be noted that, during processing, a portion of cathepsins are shunted into the exocytosis pathway without converting to the m6p form. C, Cathepsin forms that lack the signal peptide-associated alternative splicing and exon skipping-related translation targeted to the cytosol, nucleus, and mitochondrial matrix. TGN indicates trans-Golgi network.
understanding of the role of these 2 enzymes in antigen processing and presentation.

**Maturation of Cysteiny1 Cathepsin From Synthesis to Activation**

Cathepsins have been known to synthesize as proenzymes with an N-terminal signaling peptide that targets the protein to the lumen of the endoplasmic reticulum. Cathepsins destined for the lysosome are further processed in the Golgi apparatus by modification of mannose residues to mannose-6-phosphate (reviewed previously). After this modification, cathepsins bind to the mannose-6-phosphate receptor for targeting to the lysosome and to activation. Proteolytic activation is accomplished by the action of different proteases such as pepsin, neutrophil elastase, cathepsin D, and various cysteine proteases. From the results of previous studies, Reiser and colleagues proposed that active cathepsins might be recruited from late endosomes or lysosomes for secretion into the extracellular space through Ca²⁺/H⁺-dependent fusion of these organelles with the cell membrane. Limited activation is thus a crucial step in controlling the activity of cathepsins. Partial or complete removal of the proregion during activation affects the stability and folding of the enzymes. Increasing evidence indicates that the proregion plays an important role in the inhibition of cathepsin activity. The cathepsin maturation from synthesis to activation is represented schematically in Figure 1B.

**Regulation of Cysteiny1 Cathepsin**

Similar to members of the MMP family, the cathepsins are regulated at 3 levels: via the gene, activation, and activity. The activity of cathepsins can be controlled in a number of ways, two of the most important being through specific precursor activation mechanisms and through the specific regulation of mature enzymes by pH and inhibition by their endogenous protein inhibitors. It is well known that the activity of cathepsin is controlled mainly intracellularly by a specific cystatin subgroup called stefins and extracellularly by cystatins and kininogens. Significant differences will most likely occur at the N- or C-terminal parts of the prosegment. A future investigation is needed to identify the specificity determinants that allow the propeptides to differentiate between closely related members of the papain superfamily. The pH-mediated regulation has been covered by a recent comprehensive review.

In addition to the regulation of cathepsin activity level, transcriptional regulation has been studied with respect to transcriptional factors. A gene expression assay suggested that the Sp1 and Sp3 transcriptional factors binding to GC boxes can regulate several cathepsins, including cathepsins B and L. Ets family transcription factors have been linked to transcriptional regulation of cysteine cathepsins and MMPs and serine proteases (reviewed previously). On the other hand, over the past decade, there have been several reports of transcript variants arising from the use of alternative promoters and alternative splicing for cathepsins B and L. Most strikingly, recent reports suggested that alternative splicing and exon skipping can produce a complete absence of signaling peptide on cathepsins and accumulation into the nucleus and mitochondrial matrix. Truncated cathepsin L has been identified as causing loss of integrity of the glomerular filtration barrier owing to proteolysing dynamin in podocytes. In the meantime, additional cleavage substrates for cytosolic cathepsin L have been found, ie, synaptopodin and CD2AP. However, elucidation of the transcription variants and transcriptional regulation of these enzymes in cardiovascular disease requires further study. The regulation of cathepsin expression in cardiac and vascular cells is summarized in Table 1 and Figure 2.

**Cellular Expression of Cysteine Cathepsins**

Like most MMPs, neonatal cardiomyocytes express negligible levels of cathepsin S under basal conditions. However, incubation of these cells with tumor necrosis factor-α and interleukin-1β markedly augments cathepsin S gene and protein expression. These cytokines also increased the levels.
of the cathepsin K, B, and L mRNAs.22 Similar to the cardiomyocytes, both cytokines stimulated the levels of the cathepsin S and B genes in cultured cardiac fibroblasts or myofibroblasts.22,26 Furthermore, angiotensin II and superoxide markedly upregulated the levels of cathepsin S expression and activity in cultured cardiomyocytes; these changes were moderated by an antioxidative agent.24 Superoxide has also been shown to stimulate the elevation of cystatin C protein in the conditioned medium of cardiomyocytes.65 These findings suggest that cathepsins derived from cardiac cells can participate in the pathogenesis of cardiac injury in response to inflammation and oxidative stress. The expression patterns of cathepsins in cardiovascular-valve cells and CCVD-related cells are summarized in Figure 2.

Proteolytic Activities of Cysteiny1 Cathepsins

After synthesis, cathepsins are relocated to the acidic compartments, lysosomes and endosomes, through either the mannose-6-phosphate receptor–dependent or –independent pathways, where the enzymes are activated to function in unwanted substrate metabolism.48,49 These organelles give cathepsins the optimum pH for their activity.35 Our data and those of other investigators have demonstrated the presence and activity (collagenolytic and/or elastolytic) of these proteases in media conditioned by endothelial cells, smooth muscle cells, neonatal cardiomyocytes, and macrophages.6–8,66 These findings raise several questions concerning the release and extracellular activity of these proteases because most of them exit within a very narrow optimal acidic pH in the organelles.67 Previous observations suggested that cathepsins K and L can lose their activity at neutral pH.68 In contrast to these enzymes, cathepsin S has been shown to retain a pronounced elastolytic activity in the tissue extracts from the failing rat myocardium; this response was blunted by the broad-spectrum cysteine protease inhibitor epoxysuccinyl-l-leucylamido-(4-guanidino) butane (E64) or a specific inhibitor of cathepsin S, morpholinurea-leucine-homophenylalanine-

lular proteins, including cell adhesion, transmigration, differentiation, proliferation, apoptosis, and neovascularization and antigen presentation (reviewed previously67,78). Although cathepsins are abundantly present in human and animal cardiac wall22 and valve tissues,25,26,79 the exact role each specific cathepsin plays in heart disease development and the mechanism and significance behind their function are largely unknown.

Cathepsins and Cystatin C in CCVD

The pathogenesis of heart disease involves substantial proteolysis of the ventricular and valvular extracellular proteins. Different families of proteolytic enzymes may participate in this process, including MMPs, serine proteases, and cysteiny1 cathepsins. The roles of proteolytic enzymes in various cardiovascular diseases have been covered by recent comprehensive reviews.1,2

Next, we consider the role of cathepsins in CCVD in greater detail. The sections below describe the cathepsins involved in several myocardial, coronary, and valve diseases, especially with respect to their potential application as diagnostic and/or prognostic markers and drug targets to prevent CCVD.

Cathepsins in Hypertensive Cardiac Hypertrophy and Failure

The expression patterns of cathepsins in cardiovascular cells and CCVD-related cells are summarized in Figure 2. Hypertension refers to enhanced arteriole pressure and total peripheral artery resistance as a result of hemodynamic overload on the heart and is known to cause cardiac hypertrophy, fibrosis, remodeling, and heart failure (HF; reviewed elsewhere).1,80

An in vitro study reported that cathepsin is expressed in cultured rat neonatal cardiomyocytes and cardiac fibroblasts.22 The gene and protein levels of cathepsin S and/or K were markedly upregulated by tumor necrosis factor-α and interleukin-1β, which were increased in the failing rat myocardium in association with hypertension, in cultured neonatal cardiomyocytes, and in fibroblasts.22 Angiotensin II and H2O2 have also been shown to affect the expression of enzymes.24 Changes in protease expression and activity have been shown to occur in hypertension; the levels of the cathepsin S, B, and K genes have been shown to be increased in the hypertrophic and failing myocardium, whereas those of cystatin C showed no significant changes in Dahl salt-sensitive rats, a model of hypertension.22 Immunohistochemical analysis revealed only a low level of expression of cathepsins S and K in the myocardium of control rats.22 In contrast, the expression of these enzymes was markedly increased throughout the myocardium of rats with hypertensive HF, with staining apparent in cardiac myocytes, intracoronary smooth muscle cells, and dispersed macrophages. Furthermore, the elastase assays demonstrated that the levels of cathepsins S and K increased significantly with increasing elastolytic activity in the tissue extracts from the failing rat myocardium; this response was blunted by the broad-spectrum cysteine protease inhibitor trans-epoxysuccinyl-l-leucylamido-(4-guanidino) butane (E64) or a specific inhibitor of cathepsin S, morpholinurea-leucine-homophenylalanine-
Cooperation with other proteases such as MMPs and serine pate in cardiac remodeling by mediating ECM degradation in various findings support the notion that cathepsins may partici- gens.15,28 It is well known that MMPs can degrade all of the ECM proteins, including laminin,81 fibronectin,82 elastin,66 and colla- gens.15,28 It is well known that MMPs can degrade all of the ECM proteins and activate cysteine cathepsins.2 Together, these various findings support the notion that cathepsins may participate in cardiac remodeling by mediating ECM degradation in cooperation with other proteases such as MMPs and serine proteases.

**Cathepsins in Cardiomyopathy**

More than a decade ago, it was reported that defects in lysosomes and lysosomal proteases cause heterogeneous heart diseases such as cardiomyopathies.18–20 In humans, cathepsin B mRNA and protein levels are greater in dilated cardiomyopathy than in control hearts.83 Here, we have observed that the levels of cathepsins S, B, L, and/or K were increased in subjects with dilated and hypertrophic cardiomyopathies compared with control subjects (Figure 3A). Similar to cathepsins, cystatin C also showed a change in the failing myocardium of subjects with either type of cardiomyopathy. On the other hand, among cysteine cathepsins, the protein with the most extensively described role in the health and disease of the heart is cathepsin L.39 Cathepsin L is a ubiquitously expressed lysosomal cysteine protease that is primarily responsible for intracellular protein degradation.84 Genetic studies have revealed that, unlike other cathepsin-deficient (Ctsl−/−) animals, aging cathepsin L Ctsl−/− mice develop interstitial myocardial fibrosis and show pleomor- phic nuclei in cardiomyocytes; both signs are characteristic of human cardiomyopathies (Table 2).18–20 These changes are associated with cardiac chamber dilation and impaired cardiac function. Moreover, abnormal heart rhythms (supraventricular tachycardia, ventricular extrasystoles, and first-degree atrioventricular block) are detected in older Ctsl−/− mice (Table 2).19 Cardiomyocytes from Ctsl−/− newborn mice show impaired endolysosomal systems.19 Cathepsin L deficiency slows autophagolysosome turnover and results in accumulation of dysmorphic and acidic organelles.85 These findings, coupled with findings that defects in the acidic cellular compartments are accompanied by complex biochemical and mitochondrial impairment,19 raise the question of how cathepsin L deficiency and alterations of the acidic compartments change intracellular signaling to induce cardiac hypertrophic action and ventricular chamber dilation. The role of homeostatic cysteine cathepsin L in cardiomyopathy has been the subject of a review39 and therefore is not discussed in detail here.

The role of cathepsin in physiological autophagy and pathological apoptosis in cardiac disease has been investigated in several animal studies.86,87 Sehl et al87 reported that cathepsin B was overexpressed in the necrotic regions of the myocardium. It has been reported that cathepsin B participates in apoptosis of serum deprivation–induced PC12 cells. An absence of cathepsins B and L has been shown to induce

![Figure 3. Expression of cathepsin mRNAs in cardiac tissues. A. Upregulation of expression of mRNAs of cathepsins and cystatin C in patients with dilated cardiomyopathy (DCM; n=24) or hypertrophic cardiomyopathy (HCM; n=18) compared with controls (n=7). B. Representative images of increased expression of angiotensin II type 1 receptor (AT1R; rabbit polyclonal anti-AT1R) and cathepsin S (Cat S; rabbit polyclonal anti-human Cat S) and the levels of macrophage infiltration (mouse monoclonal anti rat CD68; Chemicon, Temecula, CA) and superoxide production (using dihydroethidium [DHE] staining) in the myocardium and/or intracoronary arteries with hypertensive heart failure (H-HF). Arrowheads indicate relatively positively staining cells. Values are mean±SEM. *P<0.05, **P<0.01 vs controls. C. Colocalization of Cat S and AT1R in human coronary atherosclerotic lesions. Scale bars=50 μm.](http://circ.ahajournals.org/)

Table 2. Cardiac Phenotypes of Genetically Altered Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Heart Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCtsl-TG</td>
<td>Cardiac response (in vivo aortic banding model): decreased hypertrophic response, apoptosis, and fibrosis</td>
</tr>
<tr>
<td></td>
<td>In vitro experiment: blunted cardiomyocyte hypertrophy via Akt/GSK3β signaling</td>
</tr>
<tr>
<td>Ctsl−/−</td>
<td>Intracellular (1 y old): Multiple large and fused lysosomes, storage of electron-dense heterogeneous material, and turnover of autophagolysosomes and acidic vesicles; pleomorphic nuclei; loss of cytoskeletal proteins; mitochondrial dysfunction</td>
</tr>
<tr>
<td></td>
<td>Extracellular (1 y old): interstitial fibrosis</td>
</tr>
<tr>
<td></td>
<td>Cardiac expression: cardiac chamber dilation and dysfunction, abnormal heart rhythms (SPVT, VEs, first-degree AV block)</td>
</tr>
<tr>
<td>Ctsk−/−</td>
<td>Cardiac repair (postinfarction): decreased inflammatory response and levels of G-CSF, SCF, and SDF-1 proteins, fibrosis, myofibroblast deposition, neovascularization, bone marrow cell mobilization, c-kit–positive cells, natural killer cells, fibrocytes, and monocytes in cardiac tissues</td>
</tr>
</tbody>
</table>

hCtsl-TG indicates human cathepsin L transgenic; Ctsl−/−, cathepsin L deficiency; SPVT, supraventricular tachycardia; VEs, ventricular extrasystoles; AV, atrioventricular; G-CSF, granulocyte colony stimulating factor; SCF, stem cell factor; and SDF-1, stromal-cell-derived factor-1.
neuronal loss and brain atrophy. In contrast, endogenous cystatin C inhibitor overexpression induced neuronal cell death associated with caspase-3 activation in vivo and in vitro. Furthermore, Yu et al reported that cystatin C–deficient mice exhibited increased caspase-3 expression and activity and reduced epithelial apoptosis. The antiapoptotic molecules Bcl-2, Bcl-xL, Mcl-1, and XLAP (X-chromosome-linked inhibitor of apoptosis) are targeted by the lysosomal cathepsins B and L in several human cancer cell lines. Collectively, these findings indicate that cathepsin-deficient-mediated cardiomyopathy might be attributable to the caspase-dependent and -independent apoptosis in mice. The involvement of cathepsins in autophagy and apoptosis is summarized in Table 3.

**Table 3. Cathepsin Cellular Functions and Clinical Applications**

<table>
<thead>
<tr>
<th>Cathepsins</th>
<th>Autophagy/Apoptosis</th>
<th>Biomarker Imaging Tool</th>
<th>Drug Target</th>
<th>Related Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsin B</td>
<td>+/−</td>
<td>−/+</td>
<td>−</td>
<td>Cardiac disease</td>
</tr>
<tr>
<td>Cathepsin S</td>
<td>−/−</td>
<td>+/+</td>
<td>+</td>
<td>Cardiac disease, Coronary disease</td>
</tr>
<tr>
<td>Cathepsin K</td>
<td>−/−</td>
<td>−/+</td>
<td>+</td>
<td>Cardiac disease, Coronary disease, Valve disease</td>
</tr>
<tr>
<td>Cathepsin L</td>
<td>−/+</td>
<td>+/+</td>
<td>−</td>
<td>Cardiac disease, Coronary disease, Valve disease</td>
</tr>
<tr>
<td>Cathepsin H</td>
<td>−/−</td>
<td>−/+</td>
<td>−</td>
<td>Cardiac disease</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>−/+</td>
<td>+/−</td>
<td>+</td>
<td>Cardiac disease, Coronary disease</td>
</tr>
</tbody>
</table>

+ Indicates available or reported; −, not available or no report.

It is well established that cystatin C can control cathepsin activity. Xie et al demonstrated that, in vivo, myocardial cystatin C is increased in mice that develop HF in response to hypoxic injury and that this increase is associated with local inhibition of cathepsin B activity and accumulation of collagen and fibronectin. These findings indicate the importance of the fine balance between and regulation of cathepsins and cystatins; disruption of this balance results in a pathological state caused by a deficiency or an excessive degradation of collagen and other components of the myocardial ECM. It is noteworthy that the abundance of cystatin C mRNA and protein in the left ventricular tissue does not differ between rats or humans with hypertensive HF and their respective controls. This discrepancy might be due to a differential response to ischemia and salt-induced hypertension. Understanding the importance of cystatin C in cardiac remodeling may require further experiments using conditional genetic animal models.

**Cathepsins in Coronal Artery Disease**

ECM remodeling, including collagen and elastin degradation by extracellular proteases, contributes to leaflet stiffening and valve dysfunction. The expression patterns of cathepsins in valve cells are summarized in Figure 2. Interstitial cells in myxomatous valves express excessive levels of catabolic enzymes such as cathepsins S and K, as well as MMP-1 and -13, suggesting a role of cathepsins in valve disease. This hypothesis has been confirmed in human stenotic aortic valves, which contain much greater amounts of cathepsin S, K, and V mRNAs and proteins than controls; moreover, levels of enzymatically active cathepsins are significantly higher than in controls. Recent ex vivo work demonstrates that cyclic stretch increases cathepsins S, K, and L in porcine valves, accelerating the destruction of aortic valvular ECM and the progression of aortic stenosis. Ste-notic aortic valves are characterized by atherosclerosis-like lesions containing activated inflammatory cells, calcified nodules, and bone tissue. Cathespin S deficiency lowers arterial and aortic valve calcification in ApoE−/− mice. Therefore, the role of cathepsins in promoting valve calcification may also apply to structural and functional valve degeneration; thus, they could be used in the identification of novel therapeutic targets in human valve disease.
In a study performed in the mid-1990s, it was reported that human macrophage-secreted active cathepsins S, B, and L exhibit elastolytic activity.\textsuperscript{71} More recently, both the gene and protein levels of these enzymes were found to be increased in murine diet-induced atherosclerotic plaques.\textsuperscript{105} Cathepsins S and K were the first cysteine cathepsins found to show increased protein levels in human atherosclerotic plaque when cystatin C was decreased.\textsuperscript{7,106} Increased expression of cathepsins occurs in macrophages bordering the lipid core adjacent to the fibrous cap and in macrophages and smooth muscle cells in the shoulder regions of well-developed atherosclerotic plaques, which are sites prone to rupture.\textsuperscript{6,5} It has become clear that both intracellular and extracellular activities can cause the mechanisms of action of cathepsins in atherosclerosis.\textsuperscript{107–109} The roles of cysteine cathepsins in atherosclerosis-based vascular disease, including coronary artery disease, have been covered very well by several comprehensive reviews and therefore are not discussed in detail here.\textsuperscript{42,67,78,110} However, it is noteworthy that the multiple functions of cathepsins in cardiac and valve disease have been discussed little in these reviews.

**Cathepsins Targeted by Pharmacological Interventions in CCVD**

Among synthetic cathepsin inhibitors, cathepsin K inhibitors have garnered the most interest for their potential prevention of various diseases, most notably bone resorption\textsuperscript{111} and osteoporosis.\textsuperscript{112} Nevertheless, there has been no report on their therapeutic value in CCVD. A single previous laboratory study showed that cathepsin K inhibition reduced body weight and improved glucose metabolism in mice.\textsuperscript{13} Pharmacological inhibition of cathepsin S decreased diet-induced atherosclerotic lesion formation in apolipoprotein E–deficient mice. Additionally, 1,3-transcarboxyrane2 (E64d) is a broad-spectrum inhibitor of cysteine proteases that inhibits the activity of several cathepsins (including cathepsins S, K, and B). E64d improves hypertensive cardiac hypertrophy, dysfunction, and intracoronary remodeling in the failing rat myocardium; this is accompanied by amelioration of interstitial fibrosis and of the collagen-elastic imbalance.\textsuperscript{23,24} It has been shown that, unlike deficiencies in cathepsins S\textsuperscript{108} and K,\textsuperscript{109} cathepsin L deficiency promotes organelle morphological changes and the development of interstitial fibrosis and exhibits a human cardiomyopathy-like phenotype.\textsuperscript{18–20} Although there are only limited experimental findings, the available results favor the notion that, among the cysteine cathepsins, cathepsins S and K might be the best targets of drugs to prevent cardiovascular disease in clinical trials and investigations. Collectively, the specific and nonspecific cathepsin inhibitors for preventing and treating CCVD should be tested in trials that address clinical effectiveness and whether inhibition therapies incorporate the best current medical management of CCVD.

**Circulating Cathepsins and Cystatin C as Biomarkers for CCVD**

High circulating levels of cathepsins S, L, and K and/or cystatin C have been observed in patients with atherosclerosis,\textsuperscript{119} diabetes mellitus,\textsuperscript{120} obesity,\textsuperscript{14} renal injury,\textsuperscript{121} and osteoporosis.\textsuperscript{111} Among the responsible cysteine proteases, their endogenous inhibitor, cystatin C, has garnered the most attention in HF patients with or without renal dysfunction. Cystatin C has been recognized as a sensitive marker for potential renal dysfunction and injury and an independent predictor of cardiac outcomes in patients with HF.\textsuperscript{122–125} Recent studies have suggested that serum cystatin C can reflect artery stiffness.\textsuperscript{126,127} Interestingly, data from a population-based study showed that high serum cystatin C levels were associated with increased left ventricular mass, concentric ventricular hypertrophy, and function.\textsuperscript{124} However, only recently has research been conducted to evaluate whether circulating cathepsin levels can be used as a predictive biomarker for patients with CCVD. Liu et al\textsuperscript{120} were the first to report an increase in serum cathepsin S in patients with coronary artery disease. More recently, 2 groups have reported that cathepsin L can also be used as a biomarker in coronary heart disease.\textsuperscript{119,129} Cathepsin L contributes to macrophage apoptosis and plaque destabilization in human coronary atherosclerosis.\textsuperscript{130} Shi et al\textsuperscript{106} reported a deficiency of the cathepsin inhibitor cystatin C in human atherosclerosis and aneurysm. Furthermore, evaluation of the role of cathepsins in atherosclerosis has confirmed an association between cystatin C and the progression of human coronary artery disease.\textsuperscript{131} In addition, accumulating evidence indicates the prognostic impact of circulating cathepsin...
sins B and/or L in human cancers. These findings suggest that cathepsins and/or cystatin C might be novel markers in CCVD and/or new biomarkers of therapeutic efficacy. It should be noted that no studies have evaluated circulating cathepsin levels as specific predictive biomarkers for patients with CCVD, although it appears that most cathepsins do not exhibit cell- or tissue type–specific expression patterns. A large-scale clinical cohort study with or without pharmacological interventions is needed to define the potential application of cathepsins as diagnostic and/or prognostic markers in patients with CCVD. Studies demonstrating the use of cathepsins and cystatin C as a predictive biomarkers in CCVD are summarized in Table 3.

Cathepsins as Diagnostic Imaging Tools in CCVD

Recent studies have explored the feasibility of targeting macrophage proteases for diagnostic imaging in cardiovascular inflammatory disease in vivo (Table 3 and Figure 4). It is well known that overexpression of most MMPs occurs in macrophages bordering the lipid core adjacent to the fibrous cap and in macrophages in shoulder regions of advanced atherosclerotic plaques and that this overexpression contributes critically to cardiovascular disease, particularly atherosclerosis. Therefore, intravital imaging of proteolytic activity could be applied to the evaluation of the initiation and progression of cardiovascular disease, atherosclerosis-based cardiovascular disease, and rejection of cardiac transplantation.

The MMP family has garnered the most interest as a target by dedicated imaging agents such as activatable fluorescent probes, nanoparticles, and radiolabeled inhibitors because the members of MMPs are among the most potent mammalian extracellular degradation enzymes. However, it has become clear that cathepsins can function in the extracellular space and can be targeted by imaging probes. It has been reported that cathepsin B imaging beacons are markedly activated in well-developed atherosclerotic plaques and colocalized with increased cathepsin immunoreactivity of active macrophages. Recently, the data from optical imaging with a novel protease-activatable near-infrared fluorescence probe in vivo demonstrated the preferential localization of enzymatically active cathepsin K to macrophages, consistent with their known greater elastolytic capabilities compared with vascular smooth muscle cells. More recently, intravital confocal and multiphoton microscopy and ex vivo fluorescence reflectance imaging have shown the colocalization of cathepsin S activity and calcification in aortas and aortic valves in ApoE−/−/CtsS−/− mice; Cts−/− mice show less calcification and no cathepsin S activity, validating the specificity of the probe and the involvement of the protease in this process. Furthermore, a cathepsin B near-infrared fluorescence probe was also applied to the capture of molecular information of human carotid artery atherosclerotic plaque as a complement to anatomic imaging. These
findings highlight the potential uses of the molecular probes targeting cathepsins, with the primary applications consisting of the characterization of atherosclerotic plaques in animals and humans. It is noteworthy that a single human study demonstrated that an intravascular fluorescence catheter can detect cathepsin activity in atherosclerotic plaques in vessels the size of the human coronary artery in real time with an activatable near-infrared fluorescence agent.\textsuperscript{139} This finding raises the possibility that an intravascular approach using a broad cathepsin-activatable probe can overcome the limited depth penetration of near-infrared fluorescence and be clinically useful for visualization of deep arteries in humans. New technology using cathepsin-specific probes should be tested in trials to determine its clinical usefulness, with the ultimate goal of developing a more effective noninvasive method of diagnosing cardiovascular disease and predicting its prognosis.

**Conclusions**

Many studies have investigated the role of cathepsin cysteine proteases in CCVD. Cathepsins B, K, L, and S are expressed mainly in macrophages but also in cardiovascular and valve cells and are regulated by hormones and inflammatory cytokines. Genetic studies have revealed that aged Ctsl\textsuperscript{−/−} mice develop dilated cardiomyopathy and abnormal arrhythmias. Cardiomyocytes of newborn Ctsl\textsuperscript{−/−} mice have impaired endolysosomal systems, with increased numbers of acidic organelles. Furthermore, Ctsl\textsuperscript{−/−} mice show augmented growth signaling via enhancement of the recycling of growth factors and their ligands from endosomes to the plasma membrane. The human cathepsin L transgenic heart exhibits a decrease in overload-induced cardiac hypertrophy and fibrosis through blocking of the Akt/GSK3β signaling pathway. Extracellular cathepsin L contributes to cardiac remodeling and regeneration with endothelial progenitor cell–related neovascularization. Cathepsins may be useful diagnostic tools as both imaging devices and biomarkers. In addition, a few cathepsin inhibitors are now being investigated in human trials for several diseases such as osteoporosis and rheumatoid arthritis. These chemical compounds may also affect the mechanisms underlying cardiovascular diseases. Therefore, the natural next step is to examine the efficacy of these compounds on heart and valve disease. Furthermore, because the prevalence of cardiac negative remodeling associated with ischemic and nonischemic HF increases and may coincide in the elderly, dual therapy targeting HF may be considered a future therapeutic strategy. New investigational and randomized trials are needed to determine whether selective and reversible cathepsin inhibitors will be pharmacologically effective and physiologically safe in treating human cardiovascular diseases.

**Sources of Funding**

This work was supported part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (No. 21590952 to Dr Cheng; No. 23390208 and 20249045 to Dr Murohara); the Japan Heart Foundation (No. 26-007508 to Dr Cheng); the Japan Heart Foundation/Novartis Research Award on Molecular and Cellular Cardiology (No. 26-007523 to Dr Cheng); the Scientific Research Fund of the Chinese Ministry of Education (No. 30960128 to Dr Cheng); the Japan NakATOMI Foundation (No. 26-007578 to Dr Cheng); the Japan Takeda Science Foundation (No. 26-007590 to Dr Cheng); and the National Institutes of Health (HL60942, HL81090, HL88547 to Dr Shi).

**Disclosures**

None.

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1560 Circulation March 27, 2012

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Circulation. 2012;125:1551-1562
doi: 10.1161/CIRCULATIONAHA.111.066712
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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