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[1,2]). 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, cysteinyl cathepsins were shown to localize in lysosomes and endosomes and to function there to degrade unwanted intracellular or endocytosed proteins.[3–5] 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,[6–11] obesity,[12–14] rheumatoid arthritis,[15,16] cardiac repair,[17] cardiomyopathy,[18–20] and cancer.[21] 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[22–24] and valve tissues[25–27] from humans and animals and in cultured media of cardiomyocytes,[22,24] cardiac fibroblasts,[22] vascular smooth muscle cells,[28] endothelial cells,[12] and macrophages[6,10] 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.[23,24] 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.[8,14] This review focuses on recent findings in this field, highlighting the cathepsin biology and the significance of lysosomal cysteinyl proteases in ECM remodeling, pharmacological intervention, and prediction of the development and progression of heart disease.

Biology of Cysteinyl Cathepsins

The Properties and General Structure of Cysteinyl Cathepsins

Modern molecular biology has permitted the identification of various characterization of cysteinyl protease cathepsins, including the following: (1) Cathepsins have a high homology with members of the papain family[29]; (2) they comprise mainly endopeptidases with a few exceptions (cathepsin B, exopeptidase; cathepsin H, aminopeptidase)[3,30–32]; (3) they are synthesized as glycosylated precursors that are activated by removal of the N-terminal propeptide by other proteinases or autocatalysis[33]; (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[34]; and (6) they have cell- or tissue type–specific (as in cathepsins F, S, and K)[15,35–37] and nonspecific (cathepsins B, H, and L)[38,39] 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).[40] In mice, 19 cathepsins have been discovered, including several additional placentally expressed cathepsins with no human homolog.[41] 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.[4] Human cathepsins have been shown to share a conserved active 3-dimensional pocket formed with histidine, asparagine, and cysteine residues.[21,40,42] At its N-terminus structure, cathepsin contains a small minidomain, which forms a small compact structure, and an extended peptide, which is bound

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

Cathepsins	Gene Expression Stimulatory Factors	Substrates	ECM Degradation Assay In Vitro/In Vivo
Cathepsin B	Ang II, IL-1β, TNF-α, IFN-γ	Plasminogen, antiapoptotic molecules (Bcl-2, Bcl-xL, Mcl-1, and XLAP)	−/−
Cathepsin S	Ang II, IL-1β, TNF-α, IFN-γ, VEGF, bFGF, H_2O_2	Elastin, collagen, fibronectin, laminin, MHC class II Ii	+/+
Cathepsin K	Ang II, IL-1β, TNF-α, IFN-γ	Elastin, collagen	+/+
Cathepsin L	IL-1β, TNF-α, VEGF, bFGF, glucose	Prohormones, MHC class II Ii, trypsinogen	+/+
Cystatin C	Ang II, IL-1β, TNF-α, IFN-γ, H_2O_2		+/+

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.

over the active site clef, occluding it.[39] 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.[43] 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.[29] 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.[3,44] 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[29,33,45] 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

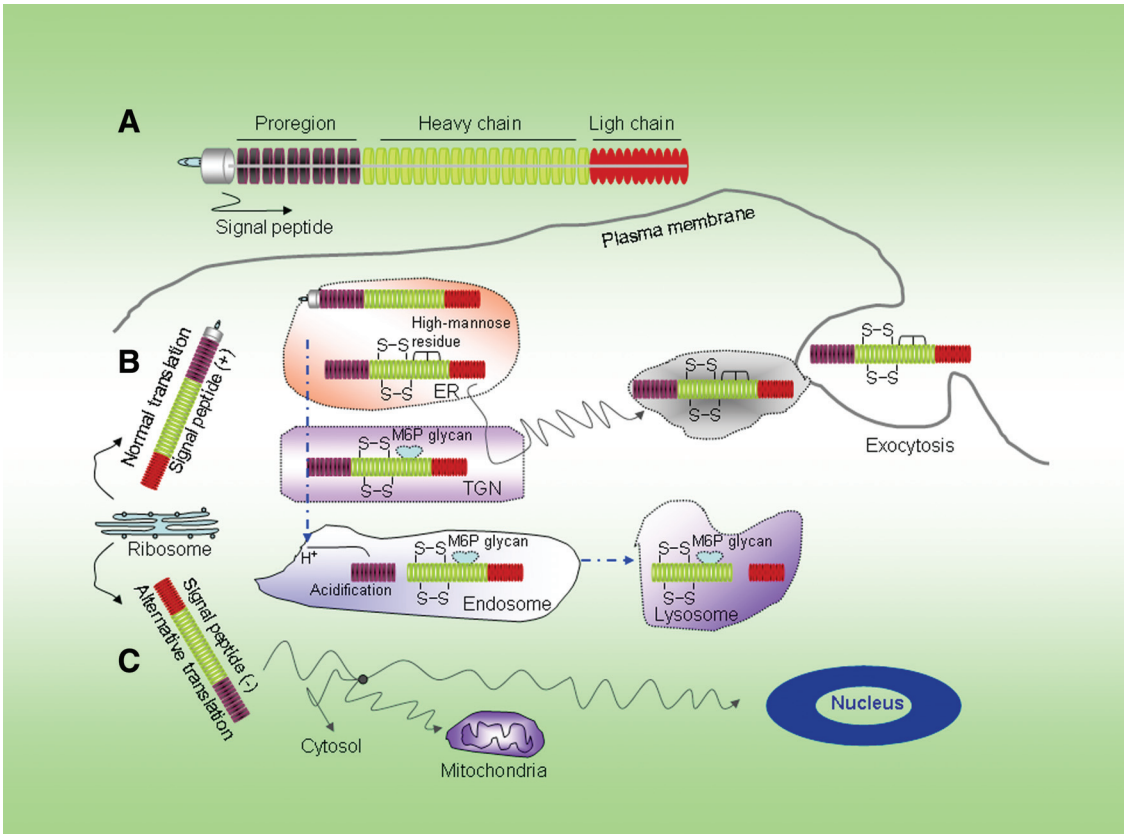

Figure 1. A model of the cathepsin primary structure and cathepsin maturation processes. **A**, Cathepsin contains a signaling peptide, proregion, heavy chain, and light chain. **B**, The cathepsin maturation process is as follows: synthesis→targeting to endoplasmic reticulum (ER)→signal peptide removing/folding 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)→Ca^{2+}-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.

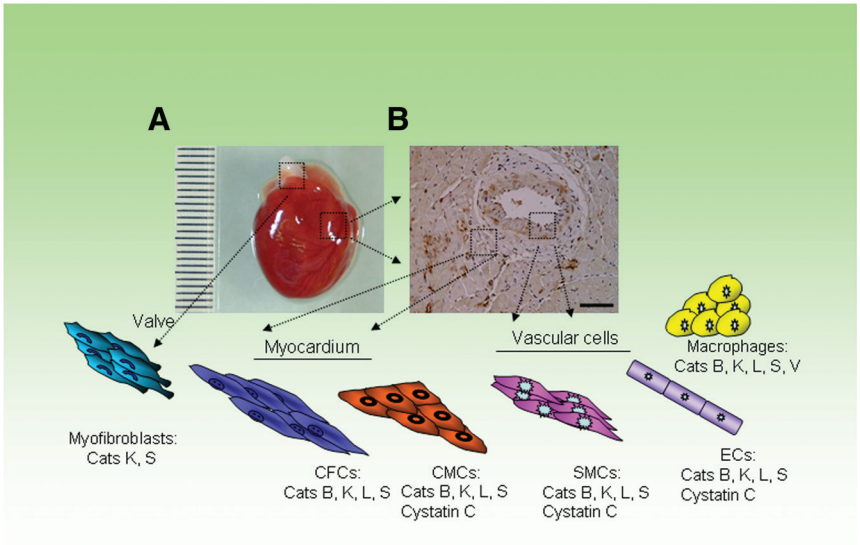

Figure 2. The cathepsin cysteine proteases known to be expressed in cardiovascular and valve cells and myocardium-coronary-valve disease (CCVD)–associated cells and have been identified as contributing to the initiation and progression of CCCVD. **A**, Macroscopy of whole heart. **B**, Microscopy of the myocardium. Scale bar=50 μm. Cats indicates cathepsins; CFC, cardiac myofibroblast; CMC, cardiomyocytes; and EC, endothelial cell.

understanding of the role of these 2 enzymes in antigen processing and presentation.

Maturation of Cysteinyl 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.[46,47] Cathepsins destined for the lysosome are further processed in the Golgi apparatus by modification of mannose residues to mannose-6-phosphate (reviewed previously[39]). After this modification, cathepsins bind to the mannose-6-phosphate receptor for targeting to the lysosome and to activation.[48,49] Proteolytic activation is accomplished by the action of different proteases such as pepsin, neutrophil elastase, cathepsin D, and various cysteine proteases.[47,50] From the results of previous studies,[51,52] Reiser and colleagues[39] proposed that active cathepsins might be recruited from late endosomes or lysosomes for secretion into the extracellular space through Ca^{2+}-dependent fusion of these organelles with the cell membrane. Limited activation is thus a crucial step in controlling the activity of cathepsins.[53] Partial or complete removal of the proregion during activation affects the stability and folding of the enzymes.[54,55] Increasing evidence indicates that the proregion plays an important role in the inhibition of cathepsin activity.[29,56] The cathepsin maturation from synthesis to activation is represented schematically in Figure 1B.

Regulation of Cysteinyl 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.[3] 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.[34] Significant differences will most likely occur at the N- or C-terminal parts of the prosegment.[33] 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.[3]

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[57] and L.[58] Ets family transcription factors have been linked to transcriptional regulation of cysteine cathepsins and MMPs and serine proteases (reviewed previously[21,57]). 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[59] and L.[21,60] Most strikingly, recent reports suggested that alternative slicing and exon skipping can produce a complete absence of signaling peptide on cathepsins and accumulation into the nucleus and mitochondrial matrix (Figure 1C).[39,60,61] Truncated cathepsin L has been identified as causing loss of integrity of the glomerular filtration barrier owing to proteolyzing dynamin in podocytes.[62] In the meantime, additional cleavage substrates for cytosolic cathepsin L have been found, ie, synaptopodin[63] and CD2AP.[64] 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.[22] 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/and 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 Cysteinyl 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 level of activity at neutral pH.[69] However, it is questionable whether this partial activity can satisfactorily explain all of the cathepsin-dependent ECM degradation observed in vitro. Recently, Punturieri et al[70] have proposed the hypothesis that the focal contact can permit cysteine proteases to degrade ECM proteins efficiently. They showed the formation of a localized acidic environment in a zone of contact that excludes the surrounding extracellular milieu in human monocyte-derived macrophages. Lysosomal H^+-ATPase has been shown to translocate across the plasma membrane and to create a localized acidic environment for lysosomal secretions in macrophages.[71] These findings provide a reasonable explanation for ECM degradation by cysteine proteases in cardiovascular tissues. Additionally, modern molecular biological techniques have allowed the characterization of novel functions of cathepsins, including a wide range of contributions to prohormone processing,[72] neuropeptide biosynthesis and secretion,[73,74] and inactivation of other proteases.[75] More recently, we have recognized that cathepsin L can play a role in cell development and differentiation via histone modification.[76,77] From these findings, we propose that both the traditional and the novel cathepsin functions work together or independently as mechanisms to contribute directly and indirectly to the initiation and progression of CCVD.

On the other hand, many cellular events in the development of atherosclerosis-based cardiovascular disease depend on the cathepsin-mediated degradation of intracellular and extracellular proteins, including cell adhesion, transmigration, differentiation, proliferation, apoptosis, and neovascularization and antigen presentation (reviewed previously[67,78]). Although cathepsins are abundantly present in human and animal cardiac wall[22] 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 cysteinyl 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 H_2O_2 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-

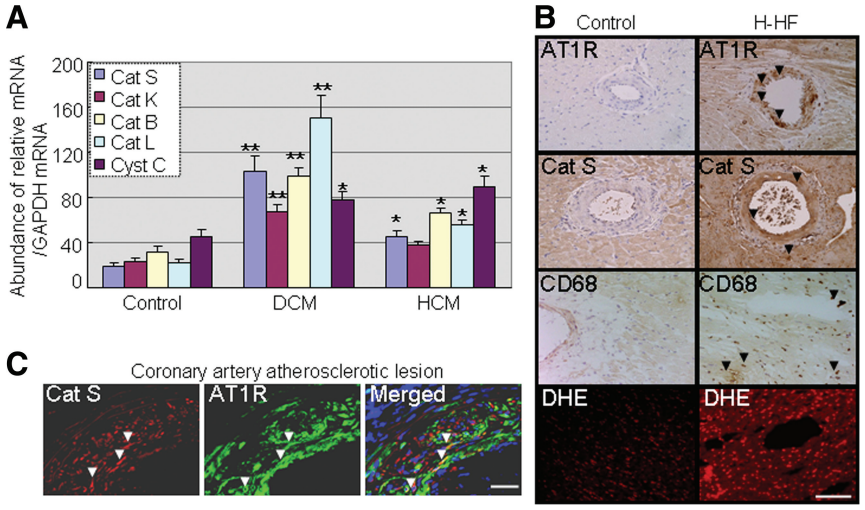

Figure 3. Expression of cathepsin mRNAs in cardiac tissues. **A**, Upregulation of expression of mRNAs of cathepsins and cystatin C in patients with dilated cardiomyopathy (DCM; n=24) or hypertrophic cardiomyopathy (HCM; n=18) compared with controls (n=7). **B**, Representative images of increased expression 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.

vinylsulfonephenyl.[22,24] Similar to the findings in the rat model, the amounts of cathepsins S and K were found to be increased in the failing myocardium of patients with hypertensive HF.[22] Active cathepsins S, K, and L have been shown to degrade ECM proteins, including laminin,[81] fibronectin,[82] elastin,[66] and collagens.[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 proteinase that is primarily responsible for intracellular protein degradation.[84] Genetic studies have revealed that, unlike other cathepsin-deficient ($Cts^{-/-}$) animals, aging cathepsin L $Ctsl^{-/-}$ mice develop interstitial myocardial fibrosis and show pleomorphic 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 review[39] 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 al[87] 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

Table 2. Cardiac Phenotypes of Genetically Altered Mice

Genotype	Heart Phenotype
hCtsl-TG	Cardiac response (in vivo aortic banding model): decreased hypertrophic response, apoptosis, and fibrosis
	In vitro experiment: blunted cardiomyocyte hypertrophy via Akt/GSK3β signaling
$Ctsl^{-/-}$	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
	Extracellular (1 y old): interstitial fibrosis
	Cardiac expression: cardiac chamber dilation and dysfunction, abnormal heart rhythms (SPVT, VEs, first-degree AV block)
$Ctsl^{-/-}$	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

h*Ctsl*-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.

Table 3. Cathepsin Cellular Functions and Clinical Applications

Cathepsins	Autophagy/ Apoptosis	Biomarker/ Imaging Tool	Drug Target	Related Disease
Cathepsin B	+/−	−/+	−	Cardiac disease
				Coronary disease
Cathepsin S	−/−	+/+	+	Cardiac disease
				Coronary disease
				Valve disease
Cathepsin K	−/−	−/+	+	Cardiac disease
				Coronary disease
				Valve disease
Cathepsin L	−/+	+/+	−	Cardiac disease
				Coronary disease
Cathepsin H	−/−	−/−	−	Cardiac disease
Cystatin C	−/+	+/−	+	Cardiac disease
				Coronary disease

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

neuronal loss and brain atrophy.[88] In contrast, endogenous cystatin C inhibitor overexpression induced neuronal cell death associated with caspase-3 activation in vivo and in vitro.[89] Furthermore, Yu et al[90] reported that cystatin C–deficient mice exhibited increased cathepsin L 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.[91] 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.

Cathepsins in Cardiac Repair

Cathepsins B, L, and H were the first to be found in the rat myocardial response to acute myocardial infarction.[92] It has been shown that cathepsin B protein levels and activity in cardiac tissues change in response to injury.[93] To our surprise, the data from Tang and colleagues[94] demonstrated in 2 hypertensive HF models (aortic banding and angiotensin II infusion) that the human cathepsin L transgenic heart shows a decrease in overload-induced cardiac hypertrophy and fibrosis through blocking of the AKT/GSK3β signaling pathway (Table 2). This was confirmed by the finding that premature signaling termination lowers the activation state of cytosolic kinases such as Akt and reduces the hypertrophic action of the challenged mouse myocardium.[94] Furthermore, *Ctsl*$^{-/-}$ mouse keratinocytes show enhanced recycling of growth factors and growth factor receptors from endosomes to the plasma membrane; this results in sustained growth signaling.[95] Moreover, in postinfarction cardiac repair, deletion of cathepsin L reduces the expression of angiogenic factors (granulocyte colony stimulating factor, stem cell factor, stromal cell–derived factor-1, and vascular endothelial growth factor) and decreases endothelial progenitor cell–related revascularization activity (Table 2).[17] Endothelial progenitor cells home to ischemic areas, differentiate, and build a framework for new blood vessels (neovascularization).[96–98] Endothelial progenitor cells show increased cathepsin L expression and activity, and infused *Cts*$^{-/-}$ progenitor cells neither home to ischemic areas nor augment ischemia-induced neovascularization.[99] Forced expression of cathepsin L in mature endothelial cells considerably enhances their invasiveness and increases their angiogenic capacity in vivo.[99] Furthermore, diabetes mellitus, a typical risk factor for ischemic heart disease, impairs human and mouse endothelial progenitor cell cathepsin L activity (but not that of other major proteases) and reduces cellular function in a glucose dose–dependent manner.[100] Hence, specific impairment of cathepsin L function by hyperglycemia may explain the poor neovascularization and regeneration capacity of ischemic tissues in diabetics.

It is well established that cystatin C can control cathepsin activity.[101] Xie et al[65] 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.[22] 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 Valve- and Atherosclerosis-Based Coronary Artery Disease

ECM remodeling, including collagen and elastin degradation by extracellular proteases, contributes to leaflet stiffening and valve dysfunction.[102,103] 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,[26] 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.[25] 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.[27] Stenotic aortic valves are characterized by atherosclerosis-like lesions containing activated inflammatory cells, calcified nodules, and bone tissue.[79] Cathepsin S deficiency lowers arterial and aortic valve calcification in *ApoE*$^{-/-}$ mice.[104] 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.[71] More recently, both the gene and protein levels of these enzymes were found to be increased in murine diet-induced atherosclerotic plaques.[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.[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.[6,9] It has become clear that both intracellular and extracellular activities can cause the mechanisms of action of cathepsins in atherosclerosis.[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.[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[111] and osteoporosis.[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.[13] Pharmacological inhibition of cathepsin S decreased diet-induced atherosclerotic lesion formation in apolipoprotein E–deficient mice. Additionally, l-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-elastin imbalance.[24] We and other groups have recently shown that E64d improves proteinuria and protects against renal injury in response to salt[113] or protamine sulfate.[114] These observations invite clinical trials of cathepsin inhibitors in cardiovascular disease. However, it should be noted that, because of the basic differences in the structures of such inhibitors, we need to develop effective chemical inhibitors to overcome their potential side effects.

There are advantages in cardiovascular drug-mediated benefits targeting cathepsins as part of the proteolytic pathway.[113,115,116] Several experimental studies have demonstrated that statins prevent diet-induced cardiac and renal injuries via the reduction of cathepsin S expression and/or activity in animal models.[113,116] It has been well established that there are closely related interactions between the renin-angiotensin signaling pathway and inflammation and NAPDH oxidase/extracellular protease activation in cardiovascular tissues.[117,118] Here, we observed that there were increases in angiotensin II type 1 receptor and cathepsin S protein expression and macrophage infiltration, as well as superoxide production, in the failing myocardium of rats with hypertension (Figure 3B) and colocalization of angiotensin receptor and cathepsin S in human coronary atherosclerotic lesions (Figure 3C). In vitro and in vivo studies have shown that angiotensin II regulates cathepsin expression and activation by enhancing NADPH oxidase–derived superoxide production.[24] The angiotensin II antagonist losartan decreases cathepsin S expression and improves advanced atherosclerotic lesion formation and atherosclerotic plaque instability.[115] Recently, it was reported that angiotensin inhibition reduced the expression of the cathepsin S and K proteins and activities and helped to improve cardiac hypertrophy, fibrosis, and dysfunction under experimental conditions without antihypertensive effects.[23,24] It has been shown that, unlike deficiencies in cathepsins S[108] and K,[109] cathepsin L deficiency promotes organelle morphological changes and the development of interstitial fibrosis and exhibits a human cardiomyopathy-like phenotype.[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,[119] diabetes mellitus,[120] obesity,[14] renal injury,[121] and osteoporosis.[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.[122–125] Recent studies have suggested that serum cystatin C can reflect artery stiffness.[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.[128] 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[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 an independent biomarker in coronary heart disease.[119,129] Cathepsin L contributes to macrophage apoptosis and plaque destabilization in human coronary atherosclerosis.[130] Shi et al[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.[131] In addition, accumulating evidence indicates the prognostic impact of circulating cathep-

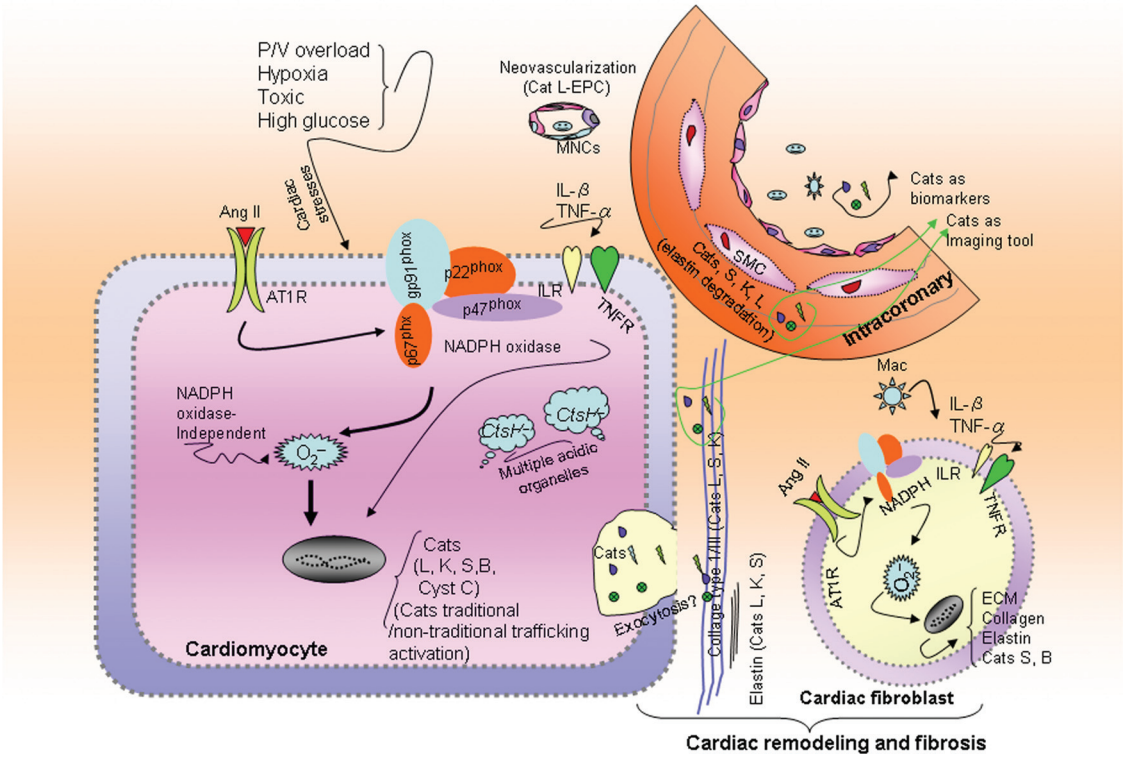

Figure 4. The roles of cathepsins (Cats) in cardiovascular remodeling and repair in response to injuries. The following information and roles of cathepsins are shown: (1) the expression and secretion of cathepsins in cardiac myocytes and fibroblasts induced by angiotensin II, inflammatory cytokines (interleukin [IL]-1β, tumor necrosis factor-α [TNF-α]), and cardiac stresses (pressure/volume [P/V] overload, hypoxia, toxic, high glucose, etc) directly or/and by their receptors; (2) the contribution of cathepsins S, K, and L to cardiac and vascular extracellular matrix (ECM) remodeling; (3) the contribution of endothelial progenitor cell (EPC)–derived cathepsin L to neovascularization in cardiac tissues in response to injury; and (4) the targeting of tissue and circulating cathepsins as biomarkers and diagnostic imaging tools for heart disease. Ang II indicates angiotensin II; AT1R, Ang II type 1 receptor; ITNR, a TNF-α receptor; ILR, IL-1β receptor; and Cyst C, cystatin C.

sins B and/or L in human cancers.[132,133] 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.[15,35,36] 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.[134,135] 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.[136,137] 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.[131] 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.[9] 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.[104] 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.[138] 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.[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 $Ctsl^{-/-}$ mice develop dilated cardiomyopathy and abnormal arrhythmias. Cardiomyocytes of newborn $Ctsl^{-/-}$ mice have impaired endolysosomal systems, with increased numbers of acidic organelles. Furthermore, $Ctsl^{-/-}$ 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.

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Disclosures

None.

References

1. Muller AL, Dhalla NS. Role of various proteases in cardiac remodeling and progression of heart failure [published online ahead of print July 8, 2011]. *Heart Fail Rev.* doi:10.1007/s10741-011-9269-8.
2. van Hinsbergh VW, Engelse MA, Quax PH. Pericellular proteases in angiogenesis and vasculogenesis. *Arterioscler Thromb Vasc Biol*. 2006; 26:716–728.
3. Turk B, Turk D, Turk V. Lysosomal cysteine proteases: more than scavengers. *Biochim Biophys Acta*. 2000;1477:98–111.
4. Turk B, Turk V, Turk D. Structural and functional aspects of papain-like cysteine proteinases and their protein inhibitors. *Biol Chem*. 1997;378: 141–150.
5. Turk B, Turk D, Salvesen GS. Regulating cysteine protease activity: essential role of protease inhibitors as guardians and regulators. *Curr Pharm Des*. 2002;8:1623–1637.
6. Sasaki T, Kuzuya M, Nakamura K, Cheng XW, Hayashi T, Song H, Hu L, Okumura K, Murohara T, Iguchi A, Sato K. AT1 blockade attenuates atherosclerotic plaque destabilization accompanied by the suppression of cathepsin S activity in apoE-deficient mice. *Atherosclerosis*. 2010; 210:430–437.
7. Sukhova GK, Shi GP, Simon DI, Chapman HA, Libby P. Expression of the elastolytic cathepsins S and K in human atheroma and regulation of their production in smooth muscle cells. *J Clin Invest*. 1998;102:576–583.
8. Liu J, Sukhova GK, Yang JT, Sun J, Ma L, Ren A, Xu WH, Fu H, Dolganov GM, Hu C, Libby P, Shi GP. Cathepsin L expression and regulation in human abdominal aortic aneurysm, atherosclerosis, and vascular cells. *Atherosclerosis*. 2006;184:302–311.
9. Jaffer FA, Kim DE, Quinti L, Tung CH, Aikawa E, Pande AN, Kohler RH, Shi GP, Libby P, Weissleder R. Optical visualization of cathepsin K activity in atherosclerosis with a novel, protease-activatable fluorescence sensor. *Circulation*. 2007;115:2292–2298.
10. Oorni K, Sneck M, Bromme D, Pentikainen MO, Lindstedt KA, Mayranpaa M, Aitio H, Kovanen PT. Cysteine protease cathepsin F is expressed in human atherosclerotic lesions, is secreted by cultured macrophages, and modifies low density lipoprotein particles in vitro. *J Biol Chem*. 2004;279:34776–34784.
11. Sun J, Sukhova GK, Zhang J, Chen H, Sjoberg S, Libby P, Xia M, Xiong N, Gelb BD, Shi GP. Cathepsin K deficiency reduces elastase perfusion-induced abdominal aortic aneurysms in mice. *Arterioscler Thromb Vasc Biol*. 2012;32:15–23.
12. Yang M, Zhang Y, Pan J, Sun J, Liu J, Libby P, Sukhova GK, Doria A, Katunuma N, Peroni OD, Guerre-Millo M, Kahn BB, Clement K, Shi GP. Cathepsin L activity controls adipogenesis and glucose tolerance. *Nat Cell Biol*. 2007;9:970–977.
13. Yang M, Sun J, Zhang T, Liu J, Zhang J, Shi MA, Darakhshan F, Guerre-Millo M, Clement K, Gelb BD, Dolgnov G, Shi GP. Deficiency and inhibition of cathepsin K reduce body weight gain and increase glucose metabolism in mice. *Arterioscler Thromb Vasc Biol*. 2008;28: 2202–2208.
14. Taleb S, Cancello R, Poitou C, Rouault C, Sellam P, Levy P, Bouillot JL, Coussieu C, Basdevant A, Guerre-Millo M, Lacasa D, Clement K. Weight loss reduces adipose tissue cathepsin S and its circulating levels in morbidly obese women. *J Clin Endocrinol Metab*. 2006;91:1042–1047.
15. Hou WS, Li W, Keyszer G, Weber E, Levy R, Klein MJ, Gravallese EM, Goldring SR, Bromme D. Comparison of cathepsins K and S expression within the rheumatoid and osteoarthritic synovium. *Arthritis Rheum*. 2002;46:663–674.
16. Asagiri M, Hirai T, Kunigami T, Kamano S, Gober HJ, Okamoto K, Nishikawa K, Latz E, Golenbock DT, Aoki K, Ohya K, Imai Y, Morishita Y, Miyazono K, Kato S, Saftig P, Takayanagi H. Cathepsin K-dependent toll-like receptor 9 signaling revealed in experimental arthritis. *Science*. 2008;319:624–627.
17. Sun M, Chen M, Liu Y, Fukuoka M, Zhou K, Li G, Dawood F, Gramolini A, Liu PP. Cathepsin-L contributes to cardiac repair and remodelling post-infarction. *Cardiovasc Res*. 2010;89:374–383.
18. Stypmann J, Glaser K, Roth W, Tobin DJ, Petermann I, Matthias R, Monnig G, Haverkamp W, Breithardt G, Schmahl W, Peters C, Rein-

heckel T. Dilated cardiomyopathy in mice deficient for the lysosomal cysteine peptidase cathepsin L. *Proc Natl Acad Sci U S A*. 2002;99:6234–6239.
19. Petermann I, Mayer C, Stypmann J, Biniossek ML, Tobin DJ, Engelen MA, Dandekar T, Grune T, Schild L, Peters C, Reinheckel T. Lysosomal, cytoskeletal, and metabolic alterations in cardiomyopathy of cathepsin L knockout mice. *FASEB J*. 2006;20:1266–1268.
20. Spira D, Stypmann J, Tobin DJ, Petermann I, Mayer C, Hagemann S, Vasiljeva O, Gunther T, Schule R, Peters C, Reinheckel T. Cell type-specific functions of the lysosomal protease cathepsin L in the heart. *J Biol Chem*. 2007;282:37045–37052.
21. Mohamed MM, Sloane BF. Cysteine cathepsins: multifunctional enzymes in cancer. *Nat Rev Cancer*. 2006;6:764–775.
22. Cheng XW, Obata K, Kuzuya M, Izawa H, Nakamura K, Asai E, Nagasaka T, Saka M, Kimata T, Noda A, Nagata K, Jin H, Shi GP, Iguchi A, Murohara T, Yokota M. Elastolytic cathepsin induction/activation system exists in myocardium and is upregulated in hypertensive heart failure. *Hypertension*. 2006;48:979–987.
23. Cheng XW, Okumura K, Kuzuya M, Jin Z, Nagata K, Obata K, Inoue A, Hirashiki A, Takeshita K, Unno K, Harada K, Shi GP, Yokota M, Murohara T. Mechanism of diastolic stiffening of the failing myocardium and its prevention by angiotensin receptor and calcium channel blockers. *J Cardiovasc Pharmacol*. 2009;54:47–56.
24. Cheng XW, Murohara T, Kuzuya M, Izawa H, Sasaki T, Obata K, Nagata K, Nishizawa T, Kobayashi M, Yamada T, Kim W, Sato K, Shi GP, Okumura K, Yokota M. Superoxide-dependent cathepsin activation is associated with hypertensive myocardial remodeling and represents a target for angiotensin II type 1 receptor blocker treatment. *Am J Pathol*. 2008;173:358–369.
25. Helske S, Syvaranta S, Lindstedt KA, Lappalainen J, Oorni K, Mayranpaa MI, Lommi J, Turto H, Werkkala K, Kupari M, Kovanen PT. Increased expression of elastolytic cathepsins S, K, and V and their inhibitor cystatin C in stenotic aortic valves. *Arterioscler Thromb Vasc Biol*. 2006;26:1791–1798.
26. Rabkin E, Aikawa M, Stone JR, Fukumoto Y, Libby P, Schoen FJ. Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodeling in myxomatous heart valves. *Circulation*. 2001;104:2525–2532.
27. Balachandran K, Sucosky P, Jo H, Yoganathan AP. Elevated cyclic stretch alters matrix remodeling in aortic valve cusps: implications for degenerative aortic valve disease. *Am J Physiol Heart Circ Physiol*. 2009;296:H756–H764.
28. Cheng XW, Kuzuya M, Nakamura K, Di Q, Liu Z, Sasaki T, Kanda S, Jin H, Shi GP, Murohara T, Yokota M, Iguchi A. Localization of cysteine protease, cathepsin S, to the surface of vascular smooth muscle cells by association with integrin alphanubeta3. *Am J Pathol*. 2006;168:685–694.
29. Coulombe R, Grochulski P, Sivaraman J, Menard R, Mort JS, Cygler M. Structure of human procathepsin L reveals the molecular basis of inhibition by the prosegment. *EMBO J*. 1996;15:5492–5503.
30. Musil D, Zucic D, Turk D, Engh RA, Mayr I, Huber R, Popovic T, Turk V, Towatari T, Katunuma N, Bode W. The refined 2.15 A x-ray crystal structure of human liver cathepsin B: the structural basis for its specificity. *EMBO J*. 1991;10:2321–2330.
31. Baici A. Inhibition of extracellular matrix-degrading endopeptidases: problems, comments, and hypotheses. *Biol Chem*. 1998;379:1007–1018.
32. Brix K, Dunkhorst A, Mayer K, Jordans S. Cysteine cathepsins: cellular roadmap to different functions. *Biochimie*. 2008;90:194–207.
33. Turk V, Turk B, Guncar G, Turk D, Kos J. Lysosomal cathepsins: structure, role in antigen processing and presentation, and cancer. *Adv Enzyme Regul*. 2002;42:285–303.
34. Dubin G. Proteinaceous cysteine protease inhibitors. *Cell Mol Life Sci*. 2005;62:653–669.
35. Chapman HA, Riese RJ, Shi GP. Emerging roles for cysteine proteases in human biology. *Annu Rev Physiol*. 1997;59:63–88.
36. Shi GP, Bryant RA, Riese R, Verhelst S, Driessen C, Li Z, Bromme D, Ploegh HL, Chapman HA. Role for cathepsin F in invariant chain processing and major histocompatibility complex class II peptide loading by macrophages. *J Exp Med*. 2000;191:1177–1186.
37. Wiederanders B, Bromme D, Kirschke H, von Figura K, Schmidt B, Peters C. Phylogenetic conservation of cysteine proteinases: cloning and expression of a cDNA coding for human cathepsin S. *J Biol Chem*. 1992;267:13708–13713.
38. Barrett AJ, Kirschke H. Cathepsin B, cathepsin H, and cathepsin L. *Methods Enzymol*. 1981;80:535–561.
39. Reiser J, Adair B, Reinheckel T. Specialized roles for cysteine cathepsins in health and disease. *J Clin Invest*. 2010;120:3421–3431.
40. Turk V, Turk B, Turk D. Lysosomal cysteine proteases: facts and opportunities. *EMBO J*. 2001;20:4629–4633.
41. Sol-Church K, Picerno GN, Stabley DL, Frenck J, Xing S, Bertenshaw GP, Mason RW. Evolution of placentally expressed cathepsins. *Biochem Biophys Res Commun*. 2002;293:23–29.
42. Qin Y, Shi GP. Cysteinyl cathepsins and mast cell proteases in the pathogenesis and therapeutics of cardiovascular diseases. *Pharmacol Ther*. 2010;131:338–350.
43. Turk D, Guncar G, Podobnik M, Turk B. Revised definition of substrate binding sites of papain-like cysteine proteases. *Biol Chem*. 1998;379:137–147.
44. Guncar G, Podobnik M, Pungercar J, Strukelj B, Turk V, Turk D. Crystal structure of porcine cathepsin H determined at 2.1 A resolution: location of the mini-chain C-terminal carboxyl group defines cathepsin H aminopeptidase function. *Structure*. 1998;6:51–61.
45. McGrath ME, Palmer JT, Bromme D, Somoza JR. Crystal structure of human cathepsin S. *Protein Sci*. 1998;7:1294–1302.
46. Kominami E, Tsukahara T, Hara K, Katunuma N. Biosyntheses and processing of lysosomal cysteine proteinases in rat macrophages. *FEBS Lett*. 1988;231:225–228.
47. Nishimura Y, Kawabata T, Kato K. Identification of latent procathepsins B and L in microsomal lumen: characterization of enzymatic activation and proteolytic processing in vitro. *Arch Biochem Biophys*. 1988;261:64–71.
48. Nishimura Y, Kawabata T, Yano S, Kato K. Inhibition of intracellular sorting and processing of lysosomal cathepsins H and L at reduced temperature in primary cultures of rat hepatocytes. *Arch Biochem Biophys*. 1990;283:458–463.
49. Kuliawat R, Klumperman J, Ludwig T, Arvan P. Differential sorting of lysosomal enzymes out of the regulated secretory pathway in pancreatic beta-cells. *J Cell Biol*. 1997;137:595–608.
50. Rowan AD, Mason P, Mach L, Mort JS. Rat procathepsin B: proteolytic processing to the mature form in vitro. *J Biol Chem*. 1992;267:15993–15999.
51. Reddy A, Caler EV, Andrews NW. Plasma membrane repair is mediated by Ca(2+)-regulated exocytosis of lysosomes. *Cell*. 2001;106:157–169.
52. Jaiswal JK, Andrews NW, Simon SM. Membrane proximal lysosomes are the major vesicles responsible for calcium-dependent exocytosis in nonsecretory cells. *J Cell Biol*. 2002;159:625–635.
53. Neurath H. Evolution of proteolytic enzymes. *Science*. 1984;224:350–357.
54. Hanewinkel H, Glossl J, Kresse H. Biosynthesis of cathepsin B in cultured normal and I-cell fibroblasts. *J Biol Chem*. 1987;262:12351–12355.
55. Cuozzo JW, Tao K, Cygler M, Mort JS, Sahagian GG. Lysine-based structure responsible for selective mannose phosphorylation of cathepsin D and cathepsin L defines a common structural motif for lysosomal enzyme targeting. *J Biol Chem*. 1998;273:21067–21076.
56. Cygler M, Sivaraman J, Grochulski P, Coulombe R, Storer AC, Mort JS. Structure of rat procathepsin B: model for inhibition of cysteine protease activity by the proregion. *Structure*. 1996;4:405–416.
57. Yan S, Berquin IM, Troen BR, Sloane BF. Transcription of human cathepsin B is mediated by Sp1 and Ets family factors in glioma. *DNA Cell Biol*. 2000;19:79–91.
58. Jean D, Guillaume N, Frade R. Characterization of human cathepsin L promoter and identification of binding sites for NF-Y, Sp1 and Sp3 that are essential for its activity. *Biochem J*. 2002;361:173–184.
59. Seth P, Mahajan VS, Chauhan SS. Transcription of human cathepsin L mRNA species hCATL B from a novel alternative promoter in the first intron of its gene. *Gene*. 2003;321:83–91.
60. Arora S, Chauhan SS. Identification and characterization of a novel human cathepsin L splice variant. *Gene*. 2002;293:123–131.
61. Muntener K, Zwicky R, Csucs G, Rohrer J, Baici A. Exon skipping of cathepsin B: mitochondrial targeting of a lysosomal peptidase provokes cell death. *J Biol Chem*. 2004;279:41012–41017.
62. Sever S, Altintas MM, Nankoe SR, Moller CC, Ko D, Wei C, Henderson J, del Re EC, Hsing L, Erickson A, Cohen CD, Kretzler M, Kerjaschki D, Rudensky A, Nikolic B, Reiser J. Proteolytic processing of dynamin by cytoplasmic cathepsin L is a mechanism for proteinuric kidney disease. *J Clin Invest*. 2007;117:2095–2104.
63. Faul C, Donnelly M, Merscher-Gomez S, Chang YH, Franz S, Delfgaauw J, Chang JM, Choi HY, Campbell KN, Kim K, Reiser J, Mundel P. The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. *Nat Med*. 2008;14:931–938.

64. Yaddanapudi S, Altintas MM, Kistler AD, Fernandez I, Möller CC, Wei C, Peev V, Flesche JB, Forst AL, Li J, Patrakka J, Xiao Z, Grahammer F, Schiffer M, Lohmüller T, Reinheckel T, Gu C, Huber TB, Ju W, Bitzer M, Rastaldi MP, Ruiz P, Tryggvason K, Shaw AS, Faul C, Sever S, Reiser J. CD2AP in mouse and human podocytes controls a proteolytic program that regulates cytoskeletal structure and cellular survival. *J Clin Invest*. 2011;121:3965–3980.
65. Xie L, Terrand J, Xu B, Tsaprailis G, Boyer J, Chen QM. Cystatin C increases in cardiac injury: a role in extracellular matrix protein modulation. *Cardiovasc Res*. 2010;87:628–635.
66. Shi GP, Sukhova GK, Kuzuya M, Ye Q, Du J, Zhang Y, Pan JH, Lu ML, Cheng XW, Iguchi A, Perrey S, Lee AM, Chapman HA, Libby P. Deficiency of the cysteine protease cathepsin S impairs microvessel growth. *Circ Res*. 2003;92:493–500.
67. Liu J, Sukhova GK, Sun JS, Xu WH, Libby P, Shi GP. Lysosomal cysteine proteases in atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2004;24:1359–1366.
68. Novinec M, Grass RN, Stark WJ, Turk V, Baici A, Lenarcic B. Interaction between human cathepsins K, L, and S and elastins: mechanism of elastinolysis and inhibition by macromolecular inhibitors. *J Biol Chem*. 2007;282:7893–7902.
69. Shi GP, Munger JS, Meara JP, Rich DH, Chapman HA. Molecular cloning and expression of human alveolar macrophage cathepsin S, an elastinolytic cysteine protease. *J Biol Chem*. 1992;267:7258–7262.
70. Punturieri A, Filippov S, Allen E, Caras I, Murray R, Reddy V, Weiss SJ. Regulation of elastinolytic cysteine proteinase activity in normal and cathepsin K-deficient human macrophages. *J Exp Med*. 2000;192:789–799.
71. Reddy VY, Zhang QY, Weiss SJ. Pericellular mobilization of the tissue-destructive cysteine proteinases, cathepsins B, L, and S, by human monocyte-derived macrophages. *Proc Natl Acad Sci U S A*. 1995;92:3849–3853.
72. Yasothornsrikul S, Greenbaum D, Medzihradszky KF, Toneff T, Bundey R, Miller R, Schilling B, Petermann I, Dehnert J, Logvinova A, Goldsmith P, Neveu JM, Lane WS, Gibson B, Reinheckel T, Peters C, Bogyo M, Hook V. Cathepsin L in secretory vesicles functions as a prohormone-processing enzyme for production of the enkephalin peptide neurotransmitter. *Proc Natl Acad Sci U S A*. 2003;100:9590–9595.
73. Hwang SR, Garza C, Mosier C, Toneff T, Wunderlich E, Goldsmith P, Hook V. Cathepsin L expression is directed to secretory vesicles for enkephalin neuropeptide biosynthesis and secretion. *J Biol Chem*. 2007;282:9556–9563.
74. Funkelstein L, Toneff T, Mosier C, Hwang SR, Beuschlein F, Lichtenauer UD, Reinheckel T, Peters C, Hook V. Major role of cathepsin L for producing the peptide hormones ACTH, beta-endorphin, and alpha-MSH, illustrated by protease gene knockout and expression. *J Biol Chem*. 2008;283:35652–35659.
75. Wartmann T, Mayerle J, Kahne T, Sahin-Toth M, Ruthenburger M, Matthias R, Kruse A, Reinheckel T, Peters C, Weiss FU, Sendler M, Lippert H, Schulz HU, Aghdassi A, Dummer A, Teller S, Halangk W, Lerch MM. Cathepsin L inactivates human trypsinogen, whereas cathepsin L-deletion reduces the severity of pancreatitis in mice. *Gastroenterology*. 2010;138:726–737.
76. Adams-Cioaba MA, Krupa JC, Xu C, Mort JS, Min J. Structural basis for the recognition and cleavage of histone H3 by cathepsin L. *Nat Commun*. 2011;2:197.
77. Duncan EM, Muratore-Schroeder TL, Cook RG, Garcia BA, Shabanowitz J, Hunt DF, Allis CD. Cathepsin L proteolytically processes histone H3 during mouse embryonic stem cell differentiation. *Cell*. 2008;135:284–294.
78. Lutgens SP, Cleutjens KB, Daemen MJ, Heeneman S. Cathepsin cysteine proteases in cardiovascular disease. *FASEB J*. 2007;21:3029–3041.
79. Balachandran K, Sucosky P, Jo H, Yoganathan AP. Elevated cyclic stretch induces aortic valve calcification in a bone morphogenic protein-dependent manner. *Am J Pathol*. 2010;177:49–57.
80. de Couto G, Ouzounian M, Liu PP. Early detection of myocardial dysfunction and heart failure. *Nat Rev Cardiol*. 2010;7:334–344.
81. Burns-Kurtis CL, Olzinski AR, Needle S, Fox JH, Capper EA, Kelly FM, McQueney MS, Romanic AM. Cathepsin S expression is up-regulated following balloon angioplasty in the hypercholesterolemic rabbit. *Cardiovasc Res*. 2004;62:610–620.
82. Taleb S, Cancello R, Clement K, Lacasa D. Cathepsin S promotes human preadipocyte differentiation: possible involvement of fibronectin degradation. *Endocrinology*. 2006;147:4950–4959.
83. Ge J, Zhao G, Chen R, Li S, Wang S, Zhang X, Zhuang Y, Du J, Yu X, Li G, Yang Y. Enhanced myocardial cathepsin B expression in patients with dilated cardiomyopathy. *Eur J Heart Fail*. 2006;8:284–289.
84. Reinheckel T, Deussing J, Roth W, Peters C. Towards specific functions of lysosomal cysteine peptidases: phenotypes of mice deficient for cathepsin B or cathepsin L. *Biol Chem*. 2001;382:735–741.
85. Dennemarker J, Lohmuller T, Muller S, Aguilar SV, Tobin DJ, Peters C, Reinheckel T. Impaired turnover of autophagolysosomes in cathepsin L deficiency. *Biol Chem*. 2010;391:913–922.
86. Yan L, Vatner DE, Kim SJ, Ge H, Masurekar M, Massover WH, Yang G, Matsui Y, Sadoshima J, Vatner SF. Autophagy in chronically ischemic myocardium. *Proc Natl Acad Sci U S A*. 2005;102:13807–13812.
87. Sehl PD, Tai JT, Hillan KJ, Brown LA, Goddard A, Yang R, Jin H, Lowe DG. Application of cDNA microarrays in determining molecular phenotype in cardiac growth, development, and response to injury. *Circulation*. 2000;101:1990–1999.
88. Felbor U, Kessler B, Mothes W, Goebel HH, Ploegh HL, Bronson RT, Olsen BR. Neuronal loss and brain atrophy in mice lacking cathepsins B and L. *Proc Natl Acad Sci U S A*. 2002;99:7883–7888.
89. Nagai A, Ryu JK, Terashima M, Tanigawa Y, Wakabayashi K, McLarnon JG, Kobayashi S, Masuda J, Kim SU. Neuronal cell death induced by cystatin C in vivo and in cultured human CNS neurons is inhibited with cathepsin B. *Brain Res*. 2005;1066:120–128.
90. Yu W, Liu J, Shi MA, Wang J, Xiang M, Kitamoto S, Wang B, Sukhova GK, Murphy GF, Orasanu G, Grubb A, Shi GP. Cystatin C deficiency promotes epidermal dysplasia in K14-HPV16 transgenic mice. *PLoS One*. 2010;5:e13973.
91. Droga-Mazovec G, Bojic L, Petelin A, Ivanova S, Romih R, Repnik U, Salvesen GS, Stoka V, Turk V, Turk B. Cysteine cathepsins trigger caspase-dependent cell death through cleavage of bid and antiapoptotic Bcl-2 homologues. *J Biol Chem*. 2008;283:19140–19150.
92. Bolli R, Cannon RO, Speir E, Goldstein RE, Epstein SE. Role of cellular proteinases in acute myocardial infarction, I: proteolysis in nonischemic and ischemic rat myocardium and the effects of antipain, leupeptin, pepstatin and chymostatin administered in vivo. *J Am Coll Cardiol*. 1983;2:671–680.
93. Crie JS, Morton P, Wildenthal K. Changes in cardiac cathepsin B activity in response to interventions that alter heart size or protein metabolism: comparison with cathepsin D. *J Mol Cell Cardiol*. 1983;15:487–494.
94. Tang Q, Cai J, Shen D, Bian Z, Yan L, Wang YX, Lan J, Zhuang GQ, Ma WZ, Wang W. Lysosomal cysteine peptidase cathepsin L protects against cardiac hypertrophy through blocking AKT/GSK3beta signaling. *J Mol Med*. 2009;87:249–260.
95. Reinheckel T, Hagemann S, Dollwet-Mack S, Martinez E, Lohmuller T, Zlatkovic G, Tobin DJ, Maas-Szabowski N, Peters C. The lysosomal cysteine protease cathepsin L regulates keratinocyte proliferation by control of growth factor recycling. *J Cell Sci*. 2005;118:3387–3395.
96. Cheng XW, Kuzuya M, Nakamura K, Maeda K, Tsuzuki M, Kim W, Sasaki T, Liu Z, Inoue N, Kondo T, Jin H, Numaguchi Y, Okumura K, Yokota M, Iguchi A, Murohara T. Mechanisms underlying the impairment of ischemia-induced neovascularization in matrix metalloproteinase 2-deficient mice. *Circ Res*. 2007;100:904–913.
97. Chavakis E, Urbich C, Dimmeler S. Homing and engraftment of progenitor cells: a prerequisite for cell therapy. *J Mol Cell Cardiol*. 2008;45:514–522.
98. Cheng XW, Kuzuya M, Kim W, Song H, Hu L, Inoue A, Nakamura K, Di Q, Sasaki T, Tsuzuki M, Shi GP, Okumura K, Murohara T. Exercise training stimulates ischemia-induced neovascularization via phosphatidylinositol 3-kinase/Akt-dependent hypoxia-induced factor-1 alpha reactivation in mice of advanced age. *Circulation*. 2010;122:707–716.
99. Urbich C, Heeschen C, Aicher A, Sasaki K, Bruhl T, Farhadi MR, Vajkoczy P, Hofmann WK, Peters C, Pennacchio LA, Abolmaali ND, Chavakis E, Reinheckel T, Zeiher AM, Dimmeler S. Cathepsin L is required for endothelial progenitor cell-induced neovascularization. *Nat Med*. 2005;11:206–213.
100. Urbich C, Dernbach E, Rossig L, Zeiher AM, Dimmeler S. High glucose reduces cathepsin L activity and impairs invasion of circulating progenitor cells. *J Mol Cell Cardiol*. 2008;45:429–436.
101. Mussap M, Plebani M. Biochemistry and clinical role of human cystatin C. *Crit Rev Clin Lab Sci*. 2004;41:467–550.
102. Helske S, Kupari M, Lindstedt KA, Kovanen PT. Aortic valve stenosis: an active atheroinflammatory process. *Curr Opin Lipidol*. 2007;18:483–491.

103. Rabkin-Aikawa E, Mayer JE Jr, Schoen FJ. Heart valve regeneration. *Adv Biochem Eng Biotechnol*. 2005;94:141–179.
104. Aikawa E, Aikawa M, Libby P, Figueiredo JL, Rusanescu G, Iwamoto Y, Fukuda D, Kohler RH, Shi GP, Jaffer FA, Weissleder R. Arterial and aortic valve calcification abolished by elastolytic cathepsin S deficiency in chronic renal disease. *Circulation*. 2009;119:1785–1794.
105. Jormsjo S, Wuttge DM, Sirsjo A, Whatling C, Hamsten A, Stemme S, Eriksson P. Differential expression of cysteine and aspartic proteases during progression of atherosclerosis in apolipoprotein E-deficient mice. *Am J Pathol*. 2002;161:939–945.
106. Shi GP, Sukhova GK, Grubb A, Ducharme A, Rhode LH, Lee RT, Ridker PM, Libby P, Chapman HA. Cystatin C deficiency in human atherosclerosis and aortic aneurysms. *J Clin Invest*. 1999;104:1191–1197.
107. Sun Y, Ishibashi M, Seimon T, Lee M, Sharma SM, Fitzgerald KA, Samokhin AO, Wang Y, Sayers S, Aikawa M, Jerome WG, Ostrowski MC, Bromme D, Libby P, Tabas IA, Welch CL, Tall AR. Free cholesterol accumulation in macrophage membranes activates Toll-like receptors and p38 mitogen-activated protein kinase and induces cathepsin K. *Circ Res*. 2009;104:455–465.
108. Sukhova GK, Zhang Y, Pan JH, Wada Y, Yamamoto T, Naito M, Kodama T, Tsimikas S, Witztum JL, Lu ML, Sakara Y, Chin MT, Libby P, Shi GP. Deficiency of cathepsin S reduces atherosclerosis in LDL receptor-deficient mice. *J Clin Invest*. 2003;111:897–906.
109. Lutgens E, Lutgens SP, Faber BC, Heeneman S, Gijbels MM, de Winther MP, Frederik P, van der Made I, Daugherty A, Sijbers AM, Fisher A, Long CJ, Saftig P, Black D, Daemen MJ, Cleutjens KB. Disruption of the cathepsin K gene reduces atherosclerosis progression and induces plaque fibrosis but accelerates macrophage foam cell formation. *Circulation*. 2006;113:98–107.
110. Lafarge JC, Naour N, Clement K, Guerre-Millo M. Cathepsins and cystatin C in atherosclerosis and obesity. *Biochimie*. 2010;92:1580–1586.
111. Stoch SA, Zajic S, Stone J, Miller DL, Van Dyck K, Gutierrez MJ, De Decker M, Liu L, Liu Q, Scott BB, Panebianco D, Jin B, Duong LT, Gottesdiener K, Wagner JA. Effect of the cathepsin K inhibitor odanacatib on bone resorption biomarkers in healthy postmenopausal women: two double-blind, randomized, placebo-controlled phase I studies. *Clin Pharmacol Ther*. 2009;86:175–182.
112. Stoch SA, Wagner JA. Cathepsin K inhibitors: a novel target for osteoporosis therapy. *Clin Pharmacol Ther*. 2008;83:172–176.
113. Cheng XW, Kuzuya M, Sasaki T, Inoue A, Hu L, Song H, Huang Z, Li P, Takeshita K, Hirashiki A, Sato K, Shi GP, Okumura K, Murohara T. Inhibition of mineralocorticoid receptor is a renoprotective effect of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor pitavastatin. *J Hypertens*. 2011;29:542–552.
114. Vassiliadis J, Bracken C, Matthews D, O'Brien S, Schiavi S, Wawersik S. Calcium mediates glomerular filtration through calcineurin and mTORC2/Akt signaling. *J Am Soc Nephrol*. 2011;22:1453–1461.
115. Suganuma E, Babaev VR, Motojima M, Zuo Y, Ayabe N, Fogo AB, Ichikawa I, Linton MF, Fazio S, Kon V. Angiotensin inhibition decreases progression of advanced atherosclerosis and stabilizes established atherosclerotic plaques. *J Am Soc Nephrol*. 2007;18:2311–2319.
116. Qin YW, Ye P, He JQ, Sheng L, Wang LY, Du J. Simvastatin inhibited cardiac hypertrophy and fibrosis in apolipoprotein E-deficient mice fed a "Western-style diet" by increasing PPAR alpha and gamma expression and reducing TC, MMP-9, and Cat S levels. *Acta Pharmacol Sin*. 2010;31:1350–1358.
117. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res*. 2000;86:494–501.
118. Landmesser U, Cai H, Dikalov S, McCann L, Hwang J, Jo H, Holland SM, Harrison DG. Role of p47(phox) in vascular oxidative stress and hypertension caused by angiotensin II. *Hypertension*. 2002;40:511–515.
119. Liu Y, Li X, Peng D, Tan Z, Liu H, Qing Y, Xue Y, Shi GP. Usefulness of serum cathepsin L as an independent biomarker in patients with coronary heart disease. *Am J Cardiol*. 2009;103:476–481.
120. Liu J, Ma L, Yang J, Ren A, Sun Z, Yan G, Sun J, Fu H, Xu W, Hu C, Shi GP. Increased serum cathepsin S in patients with atherosclerosis and diabetes. *Atherosclerosis*. 2006;186:411–419.
121. Takeuchi T, Isobe S, Sato K, Kato MI, Kasai NN, Ohyama H, Yoshikawa D, Ishii H, Matsubara T, Murohara T. Cystatin C: a possible sensitive marker for detecting potential kidney injury after computed tomography coronary angiography. *J Comput Assist Tomogr*. 2011;35:240–245.
122. Alehagen U, Dahlstrom U, Lindahl TL. Cystatin C and NT-proBNP, a powerful combination of biomarkers for predicting cardiovascular mortality in elderly patients with heart failure: results from a 10-year study in primary care. *Eur J Heart Fail*. 2009;11:354–360.
123. Arimoto T, Takeishi Y, Niizeki T, Takabatake N, Okuyama H, Fukui A, Tachibana H, Nozaki N, Hirono O, Tsunoda Y, Miyashita T, Shishido T, Takahashi H, Koyama Y, Kubota I. Cystatin C, a novel measure of renal function, is an independent predictor of cardiac events in patients with heart failure. *J Card Fail*. 2005;11:595–601.
124. Shlipak MG, Katz R, Fried LF, Jenny NS, Stehman-Breen CO, Newman AB, Siscovick D, Psaty BM, Sarnak MJ. Cystatin-C and mortality in elderly persons with heart failure. *J Am Coll Cardiol*. 2005;45:268–271.
125. Manzano-Fernandez S, Januzzi JL Jr, Boronat-Garcia M, Bonaque-Gonzalez JC, Truong QA, Pastor-Perez FJ, Munoz-Esparza C, Pastor P, Albaladejo-Oton MD, Casas T, Valdes M, Pascual-Figal DA. Beta-trace protein and cystatin C as predictors of long-term outcomes in patients with acute heart failure. *J Am Coll Cardiol*. 2011;57:849–858.
126. Madero M, Wassel CL, Peralta CA, Najjar SS, Sutton-Tyrrell K, Fried L, Canada R, Newman A, Shlipak MG, Sarnak MJ. Cystatin C associates with arterial stiffness in older adults. *J Am Soc Nephrol*. 2009;20:1086–1093.
127. Song SH, Kwak IS, Kim YJ, Lee HS, Rhee H, Lee DW, Kang YH, Kim SJ. Serum cystatin C is related to pulse wave velocity even in subjects with normal serum creatinine. *Hypertens Res*. 2008;31:1895–1902.
128. Patel PC, Ayers CR, Murphy SA, Peshock R, Khera A, de Lemos JA, Balko JA, Gupta S, Mammen PP, Drazner MH, Markham DW. Association of cystatin C with left ventricular structure and function: the Dallas Heart Study. *Circ Heart Fail*. 2009;2:98–104.
129. Zhang J, Wang P, Huang YB, Li J, Zhu J, Luo X, Shi HM, Li Y. Plasma cathepsin L and its related pro/antiangiogenic factors play useful roles in predicting rich coronary collaterals in patients with coronary heart disease. *J Int Med Res*. 2010;38:1389–1403.
130. Li W, Kornmark L, Jonasson L, Forssell C, Yuan XM. Cathepsin L is significantly associated with apoptosis and plaque destabilization in human atherosclerosis. *Atherosclerosis*. 2009;202:92–102.
131. Chen J, Tung CH, Mahmood U, Ntziachristos V, Gyurko R, Fishman MC, Huang PL, Weissleder R. In vivo imaging of proteolytic activity in atherosclerosis. *Circulation*. 2002;105:2766–2771.
132. Jain M, Bakhshi S, Shukla AA, Chauhan SS. Cathepsins B and L in peripheral blood mononuclear cells of pediatric acute myeloid leukemia: potential poor prognostic markers. *Ann Hematol*. 2010;89:1223–1232.
133. Niedergethmann M, Wostbrock B, Sturm JW, Willeke F, Post S, Hildenbrand R. Prognostic impact of cysteine proteases cathepsin B and cathepsin L in pancreatic adenocarcinoma. *Pancreas*. 2004;29:204–211.
134. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest*. 1994;94:2493–2503.
135. Cheng XW, Song H, Sasaki T, Hu L, Inoue A, Bando YK, Shi GP, Kuzuya M, Okumura K, Murohara T. Angiotensin type 1 receptor blocker reduces intimal neovascularization and plaque growth in apolipoprotein e-deficient mice. *Hypertension*. 2011;57:981–989.
136. Deguchi JO, Aikawa M, Tung CH, Aikawa E, Kim DE, Ntziachristos V, Weissleder R, Libby P. Inflammation in atherosclerosis: visualizing matrix metalloproteinase action in macrophages in vivo. *Circulation*. 2006;114:55–62.
137. Quillard T, Croce K, Jaffer FA, Weissleder R, Libby P. Molecular imaging of macrophage protease activity in cardiovascular inflammation in vivo. *Thromb Haemost*. 2011;105:828–836.
138. Kim DE, Kim JY, Schellingerhout D, Kim EJ, Kim HK, Lee S, Kim K, Kwon IC, Shon SM, Jeong SW, Im SH, Lee DK, Lee MM, Kim GE. Protease imaging of human atheromata captures molecular information of atherosclerosis, complementing anatomic imaging. *Arterioscler Thromb Vasc Biol*. 2010;30:449–456.
139. Jaffer FA, Vinegoni C, John MC, Aikawa E, Gold HK, Finn AV, Ntziachristos V, Libby P, Weissleder R. Real-time catheter molecular sensing of inflammation in proteolytically active atherosclerosis. *Circulation*. 2008;118:1802–1809.

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