Expression of cathepsin K is regulated by shear stress in cultured endothelial cells and is increased in endothelium in human atherosclerosis

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1Wallace H. Coulter Department of Biomedical Engineering at the Georgia Institute of Technology and Emory University and 2Division of Cardiology, Department of Medicine and 3Department of Surgery, Emory University School of Medicine, Atlanta; 4Atlanta Veterans Affairs Medical Center, Decatur, Georgia; and 5Department of Medicine, Harvard Medical School, Boston, Massachusetts

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Platt MO, Ankeny RF, Shi GP, Weiss D, Vega JD, Taylor WR, Jo H. Expression of cathepsin K is regulated by shear stress in cultured endothelial cells and is increased in endothelium in human atherosclerosis. Am J Physiol Heart Circ Physiol 292: H1479–H1486, 2007. First published November 10, 2006; doi:10.1152/ajpheart.00954.2006.—Cathepsins, the lysosomal cysteine proteases, are involved in vascular remodeling and atherosclerosis. Genetic knockout of cathepsins S and K in mice has shown to reduce atherosclerosis, although the molecular mechanisms remain unclear. Because atherosclerosis preferentially occurs in arteries exposed to disturbed flow conditions, we hypothesized that shear stress would regulate cathepsin K expression and activity in endothelial cells. Mouse aortic endothelial cells (MAEC) exposed to prothorogenic oscillatory shear (OS, ± 5 dyn/cm² for 1 day) showed significantly higher cathepsin K expression and activity than that of atheroprotective, unidirectional laminar shear stress (LS, 15 dyn/cm² for 1 day). Western blot and active-site labeling studies showed an active, mature form of cathepsin K in the conditioned medium of MAEC exposed to OS but not in that of LS. Functionally, MAEC exposed to OS significantly increased elastase and gelatinase activity above that of LS. The OS-dependent elastase and gelatinase activities were significantly reduced by knocking down cathepsin K with small-interfering (si) RNA, but not by a nonsilencing siRNA control, suggesting that cathepsin K is a shear-sensitive protease. In addition, immunohistochemical analysis of atherosclerotic human coronary arteries showed a positive correlation between the cathepsin K expression levels in endothelium and elastic lamina integrity. These findings suggest that cathepsin K is a mechanosensitive, extracellular matrix protease that, in turn, may be involved in arterial wall remodeling and atherosclerosis.

cathepsin K; extracellular matrix protease; shear stress; endothelial cells; vascular remodeling and atherosclerosis

CATHESPSIN K. A MEMBER OF the lysosomal cysteine protease family that was first identified in osteoclasts (1, 12, 26, 34), is a potent mammalian elastase and collagenase (1, 3, 8) and is capable of cleaving mature, insoluble elastin and collagen (1, 3, 8) as found in the arterial wall. However, cathepsin K expression and its physiological and pathophysiological role in normal blood vessels, atherosclerosis, and abdominal aortic aneurysmal development have not received much attention until recently. Cathepsins K, L, and S have been identified in atherosclerotic plaques (15, 28) and in neointima following balloon angioplasty (5). In addition, cathepsin activity is increased in abdominal aortic aneurysms (7, 15). The pathophysiological importance of cathepsin K in atherosclerosis has been demonstrated in double-knockout mice deficient in both apolipoprotein E and cathepsin K. In this atherosclerosis mouse model, the number and size of atherosclerotic lesions were reduced, whereas collagen content increased, and there were less breaks in the elastic lamina (17). Several studies have shown expression of cathepsin K by vascular smooth muscle cells (SMCs) and macrophages, and their roles in vascular remodeling (25, 28, 35), whether the endothelial expression of this enzyme plays a role in vascular remodeling and pathophysiology, has been understudied (17).

The importance of shear stress in vascular biology and pathophysiology has been highlighted by the focal development patterns of atherosclerosis in hemodynamically defined regions. For example, the regions of branched and curved arteries exposed to disturbed flow conditions, including oscillatory and low mean shear stresses [oscillatory shear (OS)], correspond to atheroprone areas. In contrast, straight arteries exposed to pulsatile, high levels of laminar shear stress (LS) are relatively well protected from atherosclerotic plaque development (36). Several human and animal studies have demonstrated that atherosclerotic lesions and aneurysms of the abdominal aorta occur in the regions where they are exposed to complex flow conditions such as flow reversal, low mean wall shear stress, and high OS index (6, 9, 21, 31–33).

We have recently shown that LS inhibits gelatinase and elastase activity in endothelial cells in a cathepsin-dependent manner. In particular, we have identified cathepsin L as one of cathepsins that partially contributes to the shear-dependent regulation of the extracellular matrix protease activity; cathepsin L knockdown showed a partial inhibitory effect (24). This clearly suggested that there were additional shear-sensitive cathepsins contributing to the matrix protease activity. Therefore, we hypothesized that shear stress would regulate cathepsin K expression and activity in endothelial cells. The current study showed that endothelial cells exposed to OS had higher cathepsin K expression and activity than that of LS, suggesting a potential role for cathepsin K at sites of disturbed flow associated with vascular pathology. In addition, studies with human atherosclerosis samples showed a positive correlation between the cathepsin K levels in endothelium and atheroscle-
rotic lesion development, providing supporting evidence for cathepsin K in atherosclerosis.

MATERIALS AND METHODS

Mouse aortic endothelial cell culture and shear stress studies. Endothelial cells obtained from the thoracic aortas of C57/BL6 control mice were cultured in growth medium [DMEM containing 10% FBS, 100 μg/ml endothelial cell growth supplement (Sigma), and 2.5 U/ml heparin] as described previously (11) and used between passages 7 and 10. Confluent endothelial monolayers grown in 100-mm tissue culture dishes were exposed to an arterial level of unidirectional LS (15 dyn/cm²) or OS with directional changes of flow at 1 Hz cycle (±5 dyn/cm²) for periods specified in the legends for Figs. 1–4 by rotating a Teflon cone (0.5°Cone angle) as described previously by us (10, 24). As a control for shear conditions, cells were treated identically for the same period as sheared cells under no flow conditions (Static). Before shear (1 h), the monolayers were washed and changed to 10 ml of fresh shear medium (the growth medium without serum).

Western blots. Following shear exposure, conditioned media were collected and normalized to 10 ml total with fresh serum-free shear media if necessary and concentrated 20–30-fold with a spin concentrator (5 kDa molecular mass cutoff; Vivascience; see Ref. 24). Cells were rinsed two times with PBS and then lysed with radioimmunoprecipitation assay buffer. Following modified Lowry protein assay, equal amounts of total protein were resolved by SDS-PAGE as described by us (13). Protein was transferred to a polyvinylidene fluoride membrane (Millipore) and probed with a mouse monoclonal anti-cathepsin K antibody (1:200; Calbiochem), anti-cathepsin L (1:500; R&D), anti-cathepsin S (1:1,000; Santa Cruz), and anti-actin (1:1,000; Santa Cruz) and goat anti-mouse IgG conjugated to alkaline phosphatase (Bio-Rad), which were detected by a chemiluminescence method (13).

Quantitative real-time PCR. Following shear exposure, cells were washed with ice-cold PBS. Real-time PCR for cathepsin K was carried out as previously described (27). Briefly, 4 μg of total RNA were reverse transcribed using random primers and a Superscript-II kit (Invitrogen) to synthesize first-strand cDNA. The cDNA was reverse transcribed using random primers and a Superscript-II kit (Invitrogen), and Taq polymerase (Invitrogen), and 25 μl of the cDNA was amplified using a LightCycler (Roche Applied Science) RT-PCR machine. The mRNA copy numbers were determined based on standard curves generated with murine cathepsin K and 18S templates. The 18S primers (50 nM each) were annealed at 61°C annealing temperature; Ambion) were used as an internal control for real-time PCR using a LightCycler and capillaries (Roche Applied Science), recombinant Taq polymerase (Invitrogen), and Taq start antibody (Clontech). A quantitative RT-PCR using cathepsin K primer pair (forward 5'-AGG TGG TTC AGA AGA TCG GAG 3'- and reverse, 5'-TCA GAC TGA ATG CCC TTC-3') was carried out using an annealing temperature of 55°C and extension time of 7 s in the PCR buffer (20 mM Tris-Cl, pH 8.4, at 25°C, 4 mM MgCl₂ to which was added 250 μg/ml BSA and 200 μM deoxynucleotides) containing SYBR green (1:84,000 dilution), 0.05 U/μl Taq DNA polymerase, and Taq Start antibody (1:100 dilution).

Transwell assay. Mouse aortic endothelial cell (MAEC) were grown to confluence on gelatin-coated Transwell filters (Corning) with pore size of 0.2 μm in growth medium. Medium (1.5 ml in the luminal and 2.5 ml in the abluminal chambers) was replaced with serum-free medium for 8 h. Equal volumes were collected from apical and basal chambers; proteins were precipitated with 5 vol of ice-cold acetone and then centrifuged at 3,000 g for 30 min; and the pellet was resuspended in a volume of lysis buffer to concentrate it 20-fold, followed by protein assay and preparation for Western blotting as described above.

Transfection of small-interfering RNA. To knock down mouse cathepsin K mRNAs, the annealed small-interfering (si) RNA duplex (sense: 5'-GCA AGC ACU GGA UAA UAA Att', antisense: 5'-UUA AUU UCG UGC UUG Ctr, MWG Biotech) and nonsilencing duplex (sense: 5'-UUC UCC GAA GUU UGC AGC Utt, antisense: 5'-ACG UGA CAC GUU CGG AGA Att; Qiagen) were obtained and used as described by us (24). Subconfluent (75–80% confluency) MAEC were transfected at a final siRNA duplex concentration of 100 nM using Oligofectamine (Invitrogen) in serum-free medium. After 6 h, the medium was supplemented with FCS (final 10% concentration) and cultured an additional 48 h before exposing the cells to OS, LS, or static conditions.

Gelatinase and elastase assay. BODIPY fluorescein-conjugated DQ elastin or gelatin (5 μg/ml; Molecular Probes) in 5 ml of fresh serum-free DMEM was incubated with MAEC following exposure to OS, LS, or static conditions for 1 day as we previously described (24). After an additional 24 h, aliquots (200 μl) of conditioned media were assayed with a fluorescence plate reader, in triplicate, with background fluorescence subtracted from the no-cell negative control at 485 nm excitation and 525 nm emission.

Active site labeling. Media was collected after 24 h exposure to OS, LS, or static conditions and centrifugally concentrated with a 5-kDa molecular mass cutoff (Vivascience). Equal aliquots of protein were incubated in buffer containing 50 mM sodium acetate, pH 4.2, 1 mM EDTA, 1% Triton X-100, and 3 mM dithiothreitol with 20 μM of the biotinylated JPM probe (16) at 37°C for 30 min before addition of reducing sample buffer, boiled, and separated by a 12.5% SDS-PAGE. Proteins were transferred to polyvinylidene difluoride membranes, and probed for blotin with the VectaStain Elite kit (Vector Labs) according to the manufacturer’s instructions.

Immunohistochemistry. Frozen sections (10 μm thick) of human coronary arteries from patients undergoing heart transplant were acquired and fixed in acetone. After being air-dried and blocked for 30 min with 1% gelatin in PBS, the cathepsin K (Calbiochem), cathepsin L (Santa Cruz), or von Willebrand factor primary antibody was incubated in buffer containing 50 mM sodium acetate, pH 4.2, 1 mM EDTA, 1% Triton X-100, and 3 mM dithiothreitol with 20 μM of the biotinylated JPM probe (16) at 37°C for 30 min before addition of reducing sample buffer, boiled, and separated by a 12.5% SDS-PAGE. Proteins were incubated with biotinylated JPM probe (16) at 37°C for 30 min before addition of reducing sample buffer, boiled, and separated by a 12.5% SDS-PAGE. Proteins were incubated with biotinylated JPM probe (16) at 37°C for 30 min before addition of reducing sample buffer, boiled, and separated by a 12.5% SDS-PAGE. Proteins were incubated with biotinylated JPM probe (16) at 37°C for 30 min before addition of reducing sample buffer, boiled, and separated by a 12.5% SDS-PAGE. Proteins were incubated with biotinylated JPM probe (16) at 37°C for 30 min before addition of reducing sample buffer, boiled, and separated by a 12.5% SDS-PAGE. Proteins were incubated with biotinylated JPM probe (16) at 37°C for 30 min before addition of reducing sample buffer, boiled, and separated by a 12.5% SDS-PAGE. Proteins were incubated with biotinylated JPM probe (16) at 37°C for 30 min before addition of reducing sample buffer, boiled, and separated by a 12.5% SDS-PAGE. Proteins were incubated with biotinylated JPM probe (16) at 37°C for 30 min before addition of reducing sample buffer, boiled, and separated by a 12.5% SDS-PAGE. Proteins were incubated with biotinylated JPM probe (16) at 37°C for 30 min before addition of reducing sample buffer, boiled, and separated by a 12.5% SDS-PAGE. Proteins were incubated with biotinylated JPM probe (16) at 37°C for 30 min before addition of reducing sample buffer, boiled, and separated by a 12.5% SDS-PAGE.

LS decreases cathepsin K protein expression in endothelial cells. Exposure of MAEC to LS significantly decreased pro (37 kDa)- and mature (25 kDa) cathepsin K protein levels in the...
cell lysates compared with that of OS and static (Fig. 1, A and B). However, MAEC cultured under static conditions contained two times as much cathepsin K as OS-stimulated cells. Because cathepsin K is known to be secreted (1), we examined whether shear regulated the protein levels in the conditioned media of MAEC. The procathepsin K protein was secreted in the conditioned media in a time-dependent manner, detectable as early as 2 h, from cells exposed to static and OS (Fig. 1B). However, procathepsin K protein level was barely detectable, if any, in the conditioned media of LS-exposed cells. After 1 day of LS exposure, procathepsin K levels in the LS group were 21% of those seen in the OS group (Fig. 1B). Interestingly, the mature form of cathepsin K was detectable only in MAEC exposed to OS, but not in those exposed to static and LS (Fig. 1B), and this is better shown with an overexposed Western blot of conditioned media collected following 24 h of shear stress (Fig. 1B, inset). We employed quantitative real-time PCR assay using 18S as an internal control to study the effect of shear stress on cathepsin K mRNA levels. MAEC exposed to OS showed 2.4- and 1.7-fold higher cathepsin K mRNA levels than those exposed to static and LS, respectively (Fig. 1C). Cathepsin K mRNA levels between static and LS were not significantly different.

Endothelial cells line the blood vessel wall and are capable of secreting proteins apically, in the bloodstream, or basally, in the vessel wall. We tested whether endothelial cells could secrete cathepsin K apically and/or basally by using a Transwell filter assay. MAECs were grown to confluence on the 0.2-

![Fig. 1. Oscillatory shear (OS) and laminar shear stress (LS) differentially regulate cathepsin K protein and mRNA expression in endothelial cells. Confluent mouse aortic endothelial cells (MAEC) were exposed to Static (St), OS, or LS for 1 day. A: representative Western blot using an equal amount of protein from cell lysates and probed with an antibody to cathepsin K (cat K) and a β-actin antibody as an internal control. Densitometric quantification of pro (37-kDa) and mature (25-kDa) forms of cathepsin K is shown as %static control in the bar graphs (means ± SE, n = 4 experiments, *P < 0.05). B: representative Western blot from the conditioned media. Recombinant cathepsin K (rCath K) was used as a positive control. The procathepsin K band was densitometrically quantified and presented as %24-h static control as shown in the bar graph (means ± SE, *P < 0.05, n = 5 at 24 h). The average is shown for 2, 4, and 8 h with n = 2 each. Inset: mature cathepsin K in a representative overexposed blot of conditioned media after 24 h in an independent study. C: following shear, total RNA from cell lysates for real-time PCR was prepared. Bar graph shows cathepsin K mRNA levels normalized against the 18S and expressed as the degree of change of static control values (means ± SE, *P < 0.05, n = 5–7). D: confluent MAEC were grown on Transwell filter, media was sampled from the apical and basal chambers, and then equal aliquots of protein were used for Western blot with the cathepsin K antibody (means ± SE, *P < 0.05, n = 5).
was apical release of the protein as well (Fig. 1C). Again, the mature cathepsin K was not detected under a static condition by Western blot. When we added recombinant mature cathepsin K to the apical or basal chamber, the protein was not detected in the opposite chamber after an 8-h incubation period (data not shown), suggesting that cathepsin K in one compartment did not simply diffuse through the cell layer to the other side.

Cathepsin K knockdown inhibits OS-sensitive elastase and gelatinase activity in endothelial cells. Because the previous results showed that cathepsin K protein expression was shear sensitive, we next examined whether cathepsin K plays a critical role in the shear-dependent extracellular matrix proteolytic activity in endothelial cells. To examine this, we used a siRNA approach to knock down cathepsin K and measured gelatinase and elastase activity. First, we showed the effectiveness and the specificity of the cathepsin K siRNA toward cathepsin K over cathepsins L and S, the other highly elastolytic and collagenolytic family members. Treatment of MAEC with cathepsin K siRNA significantly knocked down cathepsin K protein expression by 67, 60, and 56% of nonsilencing control as shown in Western blots of the cell lysates after static, OS, and LS, respectively (Fig. 2A) and 91, 75, and 87% of nonsilencing control in the conditioned media (Fig. 2B) obtained from cells exposed to static, OS, and LS, respectively. Cathepsin K siRNA was specific for cathepsin K and did not alter other members of the cathepsin family such as cathepsins L or S as determined by Western blots using both cell lysate and conditioned media (Fig. 2, A and B). It was also evident that cathepsin S was downregulated by LS in a similar fashion to cathepsin K, both intracellularly and extracellularly.

As shown in Fig. 1B, the mature form of cathepsin K in the conditioned media was not easily detectable by Western blot, but it is the form responsible for proteolytic activity. Therefore, we decided to use JPM probe, which detects the active form of cathepsins to detect active, mature cathepsin K secreted in the conditioned media. Recombinant human cathepsin K, which has a higher apparent molecular mass than the mouse form, was used as a positive control as indicated by the arrowhead in Fig. 2C. Because more than one cathepsin isoform could migrate similar to where the mouse mature cathepsin K does in one-dimensional SDS-PAGE (35), we used cathepsin K siRNA knockdown to help us identify which band would be the active cathepsin K. Figure 2C shows that the intensity of a 25-kDa band was increased by OS compared with static and LS, which confirms our findings from Fig. 1B. This is the band (arrow) in which cathepsin K siRNA specifically reduced its intensity in media from OS-exposed cells, suggesting the band represents the mature, active cathepsin K being secreted by endothelial cells in response to OS.

Under the same conditions, we tested cathepsin K knockdown effect on shear-dependent elastase and gelatinase activities. Elastase and gelatinase activity of OS and static cultured cells are much higher than that of those exposed to LS, as we have shown previously (24). Cathepsin K knockdown with siRNA reduced MAEC elastase activity by 30% in response to OS and static conditions (Fig. 3A). Cathepsin K knockdown affected gelatinase activity slightly differently. Although it significantly decreased gelatinase activity of OS-exposed cells by 43%, but it had a minimal (11%) but statistically significant effect on the static cells (Fig. 3B). As expected, LS significantly reduced the elastase and gelatinase activity in the nonsilencing controls compared with OS and static, but cathepsin K siRNA had no significant effect on extracellular matrix activities.

Endothelium overlying disrupted IEL and adjacent medial SMCs express cathepsin K. To examine the pathophysiological significance of the current in vitro findings, we carried out immunohistochemical staining with human coronary arteries using a cathepsin K antibody and evaluated IEL integrity by autofluorescence microscopy and double-blinded semiquantitation. Cathepsin K staining was most intense in the medial

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**Fig. 2.** Cathepsin K small-interfering (si) RNA knocks down cathepsin K protein and activity. Subconfluent MAECs were transfected with 100 nM of cathepsin K specific siRNA (siCat K) or nonsilencing RNA (non siRNA) 24 h before shear exposure. Transfected cells were then exposed to OS, LS, or static conditions for 1 day. Cathepsin K protein knockdown by cathepsin K siRNA was confirmed by Western blot using cell lysates (A) and conditioned media (B). Samples were probed with antibodies to cathepsins K, L, and S and a β-actin antibody as an internal control. C: active site labeling (arrows) with JPM probe by biotin blotting using the conditioned media prepared as in B and rCat K as a positive control.
layer regardless of the degree of atherosclerosis, but it was not easily detectable in the subendothelial intimal layer (Fig. 4). In contrast, endothelial cells overlying diseased plaques (Fig. 4B) did show strong expression of cathepsin K but not in minimally diseased vessels (Fig. 4A). To confirm whether cathepsin K is indeed expressed in endothelial cells, we carried out a double-staining study using a polyclonal von Willebrand factor antibody and a monoclonal cathepsin K antibody. As shown in Fig. 4I, cathepsin K and von Willebrand factor staining exactly overlapped, demonstrating that cathepsin K was indeed expressed in endothelial cells. This result is consistent with a previous report (17). Double-blinded grading of cathepsin K staining intensity and IEL integrity as determined by autofluorescence microscopy (Fig. 4, C and D) showed an apparent positive correlation ($r^2 = 0.74$) with endothelial cathepsin K expression (Fig. 4). von Willebrand factor staining of consecutive sections shows the presence of endothelium in both sections (Fig. 4, G and H), and
cathepsin L staining (Fig. 4, E and F) shows a similar expression pattern as cathepsin K.

**DISCUSSION**

The novel and significant findings of this study are that 1) OS increases endothelial cell cathepsin K mRNA and protein levels and activity compared with LS, 2) endothelial cells secrete cathepsin K preferentially toward the basal direction, 3) cathepsin K contributes significantly to shear-dependent regulation of extracellular matrix proteolytic activity in endothelial cells, and 4) expression of cathepsin K in endothelial cells showed an apparent positive correlation with disruption of IEL in human coronary arteries. These results suggest that cathepsin K is a shear-sensitive member of the cathepsin family, playing a role in extracellular matrix remodeling in the vascular wall.

The current findings suggest that cathepsin K is secreted as a pro form (Figs. 1 and 2), although it is not clear whether or not the cells secrete the mature form or if it is converted from the pro form in the conditioned media. Mature cathepsin K protein was detected by Western blot only in media from OS-exposed cells, but not that of static. Similarly, the JPM activity probe study detected more active cathepsin K in the conditioned media from OS-exposed cells than that of static (Fig. 2C). These results suggest that, unlike static and LS, OS stimulates conversion of the pro form of cathepsin K to the mature, active form in the media. This may be the reason for lower protein levels of cathepsin K in the cell lysate in the cells exposed to OS than that of static (Fig. 1A) and why OS upregulates the cathepsin K mRNA level by 2.4-fold over static controls (Fig. 1C). However, it is also likely that, because cathepsin K is activated by OS (as shown in Fig. 3C), the active protease could be capable of degrading itself, leading to a lower steady-state level of the enzyme in the cell; other cathepsins are activated by OS as well (24) and are responsible for protein turnover inside the cell.

The effect of an OS-mediated increase in cathepsin K activity, as detected by the JPM labeling study (Fig. 2C), can also be seen in the significant reduction in gelatinase activity by cathepsin K siRNA after OS exposure (Fig. 3B). Cathepsin K appears to play a greater role in gelatinolysis in cells exposed to OS than that of static cells. Cathepsin K knockdown with the siRNA showed a robust inhibitory effect (~50% reduction) in OS cells, although it had only a minor effect (~10% reduction) in the static cells. This specific OS-dependent effect on gelatinolytic activity may be because of the specific OS-activated, secreted cathepsin K identified by the JPM label and Western blots in the conditioned media (Figs. 1B and 2C). We also showed that there was no further reduction of extracellular matrix proteolytic activity under LS when cathepsin K was knocked down, presumably because LS already significantly reduced expression of cathepsin K, as shown by Western blotting and active site labeling, leaving only a minimal amount of protein to knock down further. In this study, the Western blots were performed immediately after terminating the shear stress exposure, whereas in our previous report that did not show the shear-mediated change of cathepsin K (24), the blots were performed after the cells remained in static conditions for an additional 24 h to assess gelatinase and elastase activity, by which point the cells exposed to LS had begun to secrete procathepsin K again.

It is customary to compare the effects of OS and LS with static conditions as a matter of convenience, but, in most conditions, endothelial cells cultured under static conditions do not represent the physiological environment, since they are constantly under flow in vivo. Therefore, LS is a more appropriate control for OS, and we focused on comparing the OS and LS effects in this study. The comparison between OS and LS reveals significant differences in both cathepsin K expression and activity.

**Endothelial cells are polarized cells with an apical surface exposed to the blood flowing through the lumen of the vessel and a basal portion that sits on the basement membrane and the other layers of the vessel wall. This polarity has been shown to be important in different experiments, including a previous study in our laboratory showing how caveolae form on the apical side in response to shear stress (2). Our current observation that cathepsin K is secreted preferentially out of the basal surface of the cell gives a context for its secretion in the vessel wall and suggests that shear stress could regulate cathepsin K presence and activity in vascular remodeling. Cathepsin K has been colocalized with endothelial cells in human atherosclerotic samples and with SMCs and macrophages (17). It is likely that the contribution of endothelial cell-derived cathepsin K may be primarily in the initial phases of atherosclerosis when the IEL is first degraded to allow SMC migration in the subintimal space. However, it is also possible that cathepsin K could be important in later stages of atherosclerosis and contributes to plaque rupture. In fact, transient expression of cathepsin K in human plaques has been demonstrated (17). Many plaques rupture at the shoulder regions for mechanical and biological reasons (18, 23), and the endothelium is still present at these areas. Also, fibrous plaques are composed mainly of collagen, a prime substrate for cathepsin K hydrolysis. Local secretion of cathepsin K in response to OS at these sites could be the metaphoric “straw” that breaks the plaque. Since cathepsin K can autolytically activate itself under acidic conditions (20), procathepsin K secreted into the subintimal space can be activated as macrophages in the vessel wall locally acidify the environment after coming into contact with elastin fragments (25). Atherosclerotic plaque environments also display pH heterogeneity, with lower pH found near large lipid pools, presumably because of the greater presence of lipid-laden macrophages (22). Evidence of cathepsin K at the site of plaque rupture has already been demonstrated in human samples, and the absence of cathepsin K in mice increased plaque collagen content but reduced plaque progression (17).

Here, we showed that cathepsin K was expressed in the endothelium of human coronary arteries with a correlation with the degree of IEL fragmentation (Fig. 4). The implication of this finding needs to be clarified. Although it is tempting to speculate that this enzyme is involved in breakdown of structures of the arterial wall directly or indirectly in atherosclerosis, the closer proximity of the medial cathepsin K than that produced by endothelium suggests that cathepsin K activity derived from the medial cells may be more directly involved in this process. However, unlike endothelial changes (Fig. 4), we did not find any significant difference in the intensity of the medial cathepsin K staining between the minimally diseased vessels and the atherosclerotic vessels, only changes in the
intensity of endothelial cathepsin K. In addition, transient expression of cathepsin K at different stages of the atherosclerotic development has been shown in humans (17), and these and other published images of cathepsin K staining in mouse and human atherosclerosis (17, 28) show strong cathepsin K staining near the lumen but weaker staining in the subendothelial intima. Cathepsin K secreted by endothelial cells could also contribute to the pericellular extracellular matrix proteolytic activity that destabilizes plaques by affecting the cell survival or apoptotic cues provided for the vascular and infiltrating cells (14). It may also be true that cathepsin K expression in the endothelium may help the infiltration of bloodborne leukocytes because of the increased breakdown of the subendothelial basement membrane; cathepsin S null mice were shown to have reduced macrophage and leukocyte infiltration in the subintimal space (30). Taken together, these results suggest that changes in endothelial cathepsin K levels may play an important role in this atherosclerotic process.

Serine proteases, matrix metalloproteinases (MMPs), and cysteine proteases all have members that are elastinolytic and collagenolytic, but the serine proteases and MMPs have been the focus of previous studies as the main enzymes. MMPs have even been linked to flow-mediated vascular remodeling (4, 19). Recent studies, however, have shown the important role of cathepsins and their inhibitor cystatin C in elastin degradation and atherosclerosis development. Sukhova et al. (29, 30) demonstrated that low-density lipoprotein receptor-null mice deficient in cathepsin S show decreased IEL fragmentation and reduction in atherosclerosis, whereas mice deficient for the inhibitor cystatin C had increased IEL fragmentation. With respect to this, we have previously shown that cathepsin L gelatinase and elastase activity is also regulated by shear stress in endothelial cells (24). Cathepsin L seemed to contribute more to the shear-mediated elastase activity in that study where knockdown with cathepsin L siRNA reduced it by ~50%, whereas it only reduced shear-mediated gelatinase activity by ~30% (24). In the current study, cathepsin K showed a greater involvement in OS-mediated gelatinase activity than elastase activity. Together, these two cysteine proteases have a significant impact on the shear-mediated extracellular matrix proteolytic activity of endothelial cells.

Our study provides insight into a potential mechanism by which the vascular structures and the IEL can be degraded under disturbed flow conditions. Based on our findings, we propose a following scenario: OS increases cathepsin K expression and activity in endothelial cells, which in turn stimulates gelatinase and elastase activity, whereas LS decreases this expression and activity. The increased elastase activity would then degrade elastins in the IEL, resulting in their fragmentation, subsequent arterial wall remodeling, and atherosclerotic plaque development. Elevated collagenase activity could contribute to infiltration of leukocytes and breakdown of the fibrous plaque. In summary, we showed that cathepsin K is a mechanosensitive enzyme that has a potential importance in vascular remodeling and atherosclerosis.

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