Published in final edited form as:
Future Cardiol. 2013 January ; 9(1): 89–103. doi:10.2217/fca.12.71.

Cysteine protease cathepsins and matrix metalloproteinases in the development of abdominal aortic aneurysms

Yanwen Qin[1], **Xu Cao**[1], **Yaoguo Yang**[1], and **Guo-Ping Shi**[2,*]

[1]The Key Laboratory of Remodeling-Related Cardiovascular Diseases, Beijing An Zhen Hospital, Capital Medical University, Ministry of Education, Beijing Institute of Heart, Lung & Blood Vessel Diseases, Beijing 100029, China

[2]Department of Medicine, Cardiovascular Medicine, Brigham & Women's Hospital, Harvard Medical School, 77 Avenue Louis Pasteur, NRB-7, Boston, MA 02115, USA

Abstract

Both cysteine protease cathepsins and matrix metalloproteinases are implicated in the pathogenesis of abdominal aortic aneurysms (AAAs) in humans and animals. Blood and aortic tissues from humans or animals with AAAs contain much higher levels of these proteases, and often lower levels of their endogenous inhibitors, than do blood and aortic tissues from healthy subjects. Protease- and protease inhibitor-deficient mice and synthetic protease inhibitors have affirmed that cysteinyl cathepsins and matrix metalloproteinases both participate directly in AAA development in several experimental model systems. Here, we summarize our current understanding of how proteases contribute to the pathogenesis of AAA, and discuss whether proteases or their inhibitors may serve as diagnostic biomarkers or potential therapeutic targets for this common human arterial disease.

Keywords

abdominal aortic aneurysm; cathepsins; cystatin C; matrix metalloproteinase; tissue inhibitor of matrix metalloproteinase

Abdominal aortic aneurysm (AAA) is an irreversible and fatal arterial disease, clinically defined by an aortic diameter expanded greater than 3 cm, or expanded by more than 50% of the baseline size [1]. In autopsy studies, the frequency rate of AAAs in the USA ranges from 0.5 to 3.2% [201], and there were 10,597 deaths among patients with aortic aneurysms and dissections [2]. In a recent study of 22,187 men 65 years of age, from the National Population Registry of middle Sweden, the prevalence of AAAs was 1.7% [3]. However, the exact AAA prevalence and AAA-associated mortality globally could be much higher because many countries do not have routine screening for AAAs, and individuals who die

*Author for correspondence: Tel.: +1 617 525 4358, Fax: +1 617 525 4380, gshi@rics.bwh.harvard.edu.

Financial & competing interests disclosure

This study is partially supported by grants from the National Natural Science Foundation of China (81070251, 81100222 and 81270389 to Y Qin), the United States National Institutes of Health (HL60942, HL81090 and HL88547 to G-P Shi) and the Established Investigator Award from the American Heart Association (0840118N to G-P Shi). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

from other large or small arterial diseases (e.g., atherosclerosis and stroke) may also have asymptomatic or even symptomatic AAAs. Several risk factors contribute to the increased incidence of AAAs, including age (i.e., individuals older than 55 years of age have a greatly increased risk of developing AAAs), genetic susceptibility, smoking and the presence of hypertension and/or atherosclerosis [1,4]. AAA is a complex and dynamic pathophysiological process, its molecular mechanism remains incompletely understood [5], and invasive endovascular aneurysm repair (EVAR) and open vascular surgical repair remain the only treatments [6].

AAA is an inflammatory disease characterized by extensive inflammatory cell infiltration, breakdown of the arterial extracellular matrix (ECM), neovascularization, medial smooth-muscle cell (SMC) loss, and endothelial cell (EC) death and detachment, all of which lead to aortic wall thinning and rupture. The degradation of the ECM, including elastin, collagen, laminin and fibronectin, is one of the basic pathological changes in aortic arteries in humans or animals with AAAs [5,7], and proteases secreted from inflammatory infiltrates and vascular cells exposed to inflammatory conditions play important and dominant roles in this process [8]. Many members of the cysteinyl cathepsin and matrix metalloproteinase (MMP) subfamilies are potent elastases and/or collagenases that mediate the degradation of these ECM proteins, leading to AAA expansion and rupture [9,10].

In anterior–lateral aneurysm wall tissue extracts from growing AAAs with diameters >5.5 cm and from patients with ruptured AAAs, type-I collagenase activities are mainly from neutrophil collagenase MMP-8 and cathepsins K, L and S, which are 3–30-fold higher than in extracts from normal controls [11]. Elastin degradation is a key element in controlling medial SMC loss and arterial wall thinning and rupture. Protection of this proteolysis stabilizes AAA expansion and rupture. Cathepsins S, K and L are probably the most potent mammalian elastases [12]. In peri-aortic $CaCl_2$ injury-induced AAAs in rats, elastin stabilization with pentagalloyl glucose, an elastin-binding polyphenol, may block the access of elastolytic cathepsins or MMPs, thus inhibiting elastin degradation and AAA expansion without affecting lesion calcification, macrophage content, and MMP-2 and -9 activities [13]. ECM proteolysis is also essential to neovascularization, another common feature of human AAA lesions that associates with arterial wall rupture. In human AAA lesions, the extent of neovascularization, as determined by the number of $CD31^+$ microvessels per high-power field, was significantly higher at the rupture edge from ruptured AAAs (11.4 ± 1.5; $p < 0.001$) than in the anterior sac from nonruptured AAAs (4.0 ± 0.4) or ruptured AAAs (3.7 ± 0.3) [14]. The expression of pro-angiogenic molecules, including αv-integrin, VEGF, vascular E-cadherin, MCP-1 and vimentin, was also significantly higher at the rupture edge in ruptured AAAs than in the anterior sac from nonruptured AAAs or ruptured AAAs ($p < 0.05$) [15]. Several cathepsins participate in this process [16–18], and the pro-angiogenic factor VEGF potently induces cysteinyl cathepsins and MMPs from the arterial cells [19].

Cysteinyl cathepsin expression regulation & activity

Cysteinyl cathepsins belong to the papain subfamily of the cysteine protease family, with their highly homologous primary amino acid sequences at the active site regions and their closely related physiological and pathological activities [20,21]. There are 11 members in this family in humans, including cathepsins B, C, F, H, K, L, O, S, V, W and X [22]. These proteases retain their activities under acidic pH, reside in the late endosomes and lysosomes to degrade unwanted endocytosed proteins, and act as ‘housekeeping’ enzymes. Their sensitivity to pH may regulate their activities, but cystatins are probably the most common regulators of cysteinyl cathepsins. As endogenous inhibitors, cystatins control cysteinyl cathepsin activity, and even their synthesis [23]. Although cathepsins are largely considered as lysososmal proteases, they also appear in the cytosol, cell membrane and extracellular

space. We do not know how cathepsins are sent to the cell membrane or are secreted to the extracellular space. Angiotensin II (Ang II), for example, enhances lysosomal cathepsin F (CatF) secretion in monocyte-derived macrophages [24]; cathepsins seem to be released into the cytoplasm through lysosomal membrane permeabilization or calpain-dependent lysosomal membrane damage [25], where cathepsins play important roles in apoptosis and necrosis [26].

SMCs, ECs and macrophages are essential components of the vasculature; their homeostatic gene expression is important in maintaining vascular wall integrity. Our early studies revealed a broad view of vascular cell protease expression profiles under inflammatory conditions, critical to inflammation-associated vascular tissue remodeling [19]. Cultured human SMCs displayed no immunoreactive cathepsins K and S, and exhibited little or no elastolytic activity when incubated with insoluble elastin. SMCs stimulated with the proinflammatory cytokines IL-1β or IFN-γ secreted active CatS to degrade extracellular insoluble elastin. A selective small molecule inhibitor of CatS blocked >80% of this elastinolytic activity [27].

In addition to ECM degradation [22,28], growing evidence shows that lysosomal cathepsins play important roles in cell apoptosis [22,29]; cell signaling [30,31]; antigen presentation and T-cell activation [32]; angiogenesis [27,18,33]; matrix protein gene expression; and pro-enzyme, latent cytokine, chemokine and growth factor activation [10]. Intracellular cathepsins induce cell apoptosis through activation of the Bid/Bax pathway [25]. CatS promotes angiogenesis by generating pro-angiogenic peptides from ECM laminin-5 and clearing anti-angiogenic peptides generated from collagen IV proteolysis [18]. All of these activities suggest cathepsin participation in the pathogenesis of atherosclerosis [34], myocardial remodeling [35], pneumonia [36], arthritis [37], allergic reactions [38], cancer [39] and Alzheimer's disease [40]. Pharmacological inhibition of cathepsins may alleviate the progress of these diseases [41]. The role of cathepsins in cardiovascular diseases has raised concern worldwide in recent years [10]. ECM remodeling is one of the underlying mechanisms in cardiovascular diseases [42]. Inflammatory cytokines, such as TNF-α, and monocyte binding stimulate cathepsin expression and activity from ECs [43]. These steps may initiate local proteolysis as part of AAA pathogenesis. Imbalance between cysteinyl cathepsins and their endogenous inhibitors dysregulates arterial integrity and enhances arterial remodeling during aortic aneurysm formation [33,44].

Cathepsin expression & function in human & experimental AAAs

When normal arteries contain little or no CatK or CatS, macrophages, SMCs and ECs in human AAA lesions contain abundant immunoreactive cathepsins K and S [27]. In a recent randomized population-based study, we found, using Student's t test, that plasma total (14.7 ± 4.25 vs 10.7 ± 3.67 ng/ml; $p < 0.001$), active (11.0 ± 3.70 vs 7.78 ± 3.37 ng/ml; $p < 0.001$) and pro-CatS (3.74 ± 2.00 vs 3.04 ± 2.19 ng/ml; $p < 0.001$) levels were significantly higher in 476 male AAA patients than in 200 age-matched male controls. A logistic regression test suggested that plasma total (odds ratio [OR]: 1.332), active (OR: 1.21) and pro-CatS (OR: 1.25) levels were independent AAA risk factors that associated positively with AAAs ($p < 0.001$). By contrast, plasma cystatin C levels associated significantly, but negatively, with AAAs (OR: 0.356; $p < 0.001$) [45]. These observations suggested further that CatS, CatK and possibly other cathepsins participate in human AAA formation.

Several mechanistic insights of cathepsin functions in AAAs have been revealed after the generation and in-depth analysis of cathepsin gene knockout and transgenic AAA mice. At least four cysteinyl cathepsins have been tested in AAA formation – CatS [46], CatK [47,48], CatL [16,49] and CatC [50]. Using Ang II infusion-induced experimental AAAs in

apolipoprotein E-deficient (*Apoe*$^{-/-}$) mice, we recently demonstrated increased CatS expression in mouse AAA lesions. Absence of CatS significantly reduced AAA incidence and aortic diameters. Mechanistic characterization showed that CatS deficiency improved arterial wall elastin integrity and collagen accumulation, and reduced lesion CatK and MMP-2 expression, SMC loss, overall AAA lesion cell apoptosis, angiogenesis, macrophage and $CD4^+$ T-cell migration and accumulation, and lesion cell proliferation [46]. CatK functions in AAAs have been assessed with both aortic elastase perfusion and systemic Ang II infusion in *Apoe*$^{-/-}$ mice. Using porcine pancreatic elastase aortic perfusion-induced AAA in mice, we showed that mice lacking CatK (*Ctsk*$^{-/-}$) developed significantly smaller AAAs at 14 days after elastase perfusion. CatK deficiency did not affect AAA lesion macrophage content, but reduced lesion $CD4^+$ T-cell content and proliferation, and lesion overall and media SMC apoptosis, thereby protecting the arterial wall from SMC loss. Through unknown mechanisms, lack of CatK reduced lesion activities of CatL, MMP-2 and MMP-9, but did not significantly affect lesion angiogenesis [47]. By contrast, Bai *et al.* reported that CatK deficiency did not affect AAA lesions in *Apoe*$^{-/-}$ mice induced by Ang II infusion [48]. Although the discrepancies between the two studies remain unexplained, we noticed that *Ctsk*$^{-/-}$ mice had increased Ang II-induced peripheral $CD4^+CD25^+$ T cells and $Ly6^+$ leukocytes. We suspected that the discrepancies were largely due to Ang II perfusion [47]. CatL is also highly expressed in SMCs, ECs and macrophages in human AAA lesions, and its expression in the vascular cell types found in these lesions is regulated by proinflammatory cytokines [49]. Using aortic elastase perfusion-induced experimental AAAs and peri-aortic $CaCl_2$ injury-induced aorta expansion in mice, we demonstrated that CatL deficiency protected mice from AAA formation [16]. Absence of CatL reduced AAA lesion inflammatory cell (macrophages and $CD4^+$ T cells) and chemokine MCP-1 content, lesion angiogenesis, cell proliferation and medial elastin degradation, but did not affect lesion cell apoptosis. Similar to the findings in *Ctsk*$^{-/-}$ mice, AAA lesions or SMCs from *Ctsl*$^{-/-}$ mice showed reduced expression and activities of cathepsins B and K, and MMP-1, -2, -3 and -9 [16]. CatC, also called dipeptidyl peptidase I, mediates the activation of serine proteases, including neutrophil elastase, CatG and proteinase 3. CatC deficiency also protected mice from aortic elastase perfusion-induced AAA formation by reducing neutrophil recruitment to AAA lesions [50]. Table 1 summarizes different roles of all four cathepsins that have been tested in experimental AAAs.

Not all cathepsins are cysteine proteases. CatA and CatG, for example, are serine proteases, and CatD and CatE are aspartate proteases [22]. CatD and CatA have been implicated in AAA formation. Plasma CatD levels are significantly higher in AAA patients than in control subjects [51]. CatA activity from a parietal thrombus homogenate of an aneurysm is much higher than in a blood clot homogenate [52], but direct evidence of their participation in AAA formation is currently not available.

Cystatin C expression & function in AAAs

Cystatin C is the most abundant endogenous inhibitor of cysteinyl cathepsins and is ubiquitously expressed in almost all tested cells. Its expression is reduced or deficient in human AAAs and atherosclerotic lesions [53]. Serum cystatin C levels were significantly lower in patients with AAAs than in non-AAA controls [53,54]. Serum cystatin C levels correlated negatively with AAA size and annual expansion rate, persisting after adjustment for renal function, smoking, diastolic blood pressure, C-reactive protein (CRP), age and AAA size. Cystatin C deficiency was associated with increased aneurysm size and expansion rate, possibly owing to a lack of inhibition of cysteine proteases [45,54]. *In vitro*, cytokine-stimulated vascular SMCs secrete cathepsins, whose elastolytic activity could be blocked when cystatin C secretion was induced by treatment with TGF-β. These findings

highlight an imbalance between cysteinyl cathepsins and cystatin C during arterial wall remodeling [53]. We tested a role for cystatin C in aortic expansion and AAAs. Atherosclerosis-prone *Apoe*$^{-/-}$ mice developed atherosclerosis after consuming 12 weeks of an atherogenic diet. Absence of cystatin C increased aortic SMC and aortic arch cathepsin activities, enhanced aortic arch medial elastin degradation and enlarged abdominal aortic circumference in *Apoe*$^{-/-}$ mice [55]. In Ang II infusion-induced experimental AAAs in *Apoe*$^{-/-}$ mice, deficiency of cystatin C increased AAA lesion areas, external diameters and luminal diameters. Lesion characterization showed increased AAA lesion macrophages, T cells and adhesion molecule (VCAM-1) expression, elevated medial elastin degradation and SMC loss, and enhanced lesion cathepsin activities and angiogenesis [56].

Human and animal studies have provided direct and indirect evidence to support a role of cysteinyl cathepsins in AAA formation. Clinical evaluation of plasma cysteinyl cathepsins and cystatin C suggest that they may serve as diagnostic biomarkers [9,45,57,58] or therapeutic targets. Although all available data from experimental atherosclerosis and AAAs indicate essential roles for cathepsins in these vascular diseases, no inhibitors in this category have been used in humans.

MMPs & their expression & regulation in AAA lesions

MMPs are zinc-dependent endopeptidases and belong to a larger family of proteases known as the metzincin superfamily. MMPs were first described as proteases that act on protein components of the ECM [59], but they also process a number of bioactive molecules, including cell surface receptors, apoptotic ligands (such as the FAS ligand), chemokines, cytokines, growth factors and proteases [60–66]. MMPs therefore also contribute to cell behaviors such as cell proliferation, migration (adhesion and dispersion), differentiation, angiogenesis, apoptosis and host defense. MMPs are important in various physiological and pathological processes, including morphogenesis, angiogenesis, tissue repair, cirrhosis, arthritis and tumor metastasis.

In *Apoe*$^{-/-}$ mice, Ang II infusion induces AAA formation, and aortic tissue MMP-2 and MMP-9 activities (as determined by gelatin zymography) increase over time [67]. In human AAA lesions, increased expression of interstitial collagenase MMP-1 was found in mesenchymal cells, whereas collagenases MMP-3 and -9 were expressed in mononuclear cells or macrophages [68]. Leukocytes [69] and macrophages in AAAs [68], for example, are major sources of MMP-9. Compared with non-aneurysmal aortas with arteriosclerotic occlusive disease (AOD) and normal aortic tissues, human AAA tissues contain significantly increased levels of matrix-bound 72-kDa type IV collagenase MMP-2, active MMP-2 and MMP-2-activating protease MT-1 (membrane type-1) MMP [70,71], one of the dominant type I collagenases in the human vasculature [72]. Aortic tissue active (4.5 ng/mg protein vs 0.5 ng/mg protein; $p < 0.001$) and total MMP-8 levels (16.6 ng/mg protein vs 2.8 ng/mg protein; $p < 0.001$), a major AAA lesion collagenase [11], are significantly higher in human AAA lesions than in normal aortas [73]. Gelatin gel zymography showed higher MMP-9 activities in ruptured AAAs (2647 ± 498 arbitrary unit/mg protein) than in medium-sized AAAs (5 cm < diameter < 7 cm, 1907 ± 247 arbitrary unit/mg protein), and significantly more than in large AAAs (diameter ≥7 cm, 1190 ± 247 arbitrary unit/mg; $p < 0.05$) [74]. Zymography and immunoblot analysis demonstrated increased expression of MMP-2 in small AAA lesions and MMP-9 in larger AAA lesions [75]. In an organ culture study, tissue explants from AAA patients produced 500-fold more MMP-9 (3218.5 ± 1115.2 ng/g) than normal aortas (6.14 ± 2.3 ng/g; $p < 0.001$) [76]. Collagenase MMP-13 mRNA (determined by RT-PCR) and protein levels were also increased in human AAA lesions [77].

In AAA lesions, infiltrated inflammatory cells can be a major source of increased MMP levels. These inflammatory cells – including macrophages, neutrophils, mast cells, T cells and B cells – increased during AAA progression, either with greater infiltration or proliferation [78]. Inhibition of inflammatory cell accumulation or proliferation to the AAA lesion may reduce MMP expression, thereby delaying or ablating AAA development. In Ang II infusion-induced AAA lesions in *Apoe*$^{-/-}$ mice, leukotriene B4 (LTB4) expression increased at 4 weeks after Ang II infusion. Absence of LTB4 receptor BLT1 reduced AAA incidence, suprarenal to infrarenal aortic diameter ratio, and total suprarenal to infrarenal area ratio. As expected, lesions from *Blt1*$^{-/-}$ mice had significantly reduced AAA lesion Mac-3-positive macrophages, and chemokine expression, including MCP-1, MIP-1α and MIP-2 [79]. In addition to chemokines, such as IL-8, MIP-1α and MCP-1 [78], ECM degradation-released elastin fragments also mediate inflammatory cell infiltration to AAA lesions [75]. These cells not only produce MMPs, but also release inflammatory cytokines, including TNF-α, IL-1β and IL-6, to regulate inflammatory and mesenchymal cell MMP production [19,80]. Depletion of inflammatory cells, such as neutrophils [81] and mast cells [82], reduced AAA formation in mice.

Association of circulating MMPs with human AAAs

Although several studies have shown no correlation of plasma MMP levels with aortic dilation [83], or between AAA and AOD patients [84], most studies have demonstrated significantly higher plasma MMP levels in AAA patients, compared with AOD patients or healthy subjects. Watanabe *et al.*, for example, showed that AAA patients had serum MMP-9 levels of 622.0 ± 400.2 ng/ml, whereas AOD patients and healthy controls had levels of only 284.3 ± 151.4 ng/ml and 280.8 ± 165.5 ng/ml ($p < 0.001$), respectively. Furthermore, they demonstrated that serum MMP-9 levels in AAA patients who underwent surgical repairs dropped to the levels of normal subjects (268.1 ± 215.9 ng/ml) [85]. In a separate study by Hovsepian *et al.*, AAA patients had 99.4 ± 17.4 ng/ml serum MMP-9, whereas AOD and normal control subjects had 36.1 ± 7.7 ng/ml and 54.7 ± 10.5 ng/ml, respectively [86]. A comparable study by McMillan and Pearce showed that AAA patients also had significantly higher serum MMP-9 (85.66 ± 11.64 ng/ml) than AOD patients (25.75 ± 4.125 ng/ml; $p < 0.001$) or healthy controls (13.16 ± 1.94 ng/ml; $p < 0.001$) [76]. Among EVAR patients, those with endoleak have significantly higher levels of plasma MMP-9 levels than those without endoleak (89.54 ± 26.46 vs 25.02 ± 13.40 ng/ml; $p < 0.001$). Regression analysis showed no significant influence of age, sex and AAA sizes [87]. In a population-based study from the Dallas Heart Study, higher plasma MMP-9 levels independently associated with higher aortic wall thickness ($p < 0.0001$) and larger luminal diameter ($p < 0.0001$) [88].

In plasma, both MMP-1 and -9 levels were significantly higher in patients with ruptured AAAs than in patients with nonruptured AAAs [89]. Among ruptured AAAs, pre-operative plasma MMP-9 levels were also much higher in patients who did not survive at 30 days post-surgery than in those who survived [89]. In AAA patients, MMP-8 ($p < 0.001$) and MMP-9 ($p = 0.01$) were more significantly elevated in aortic tissues than in tissues from the anterior aneurysm wall [90]. Most of these human studies suggest that MMPs participate in AAA formation, or at least that plasma MMP levels correlate with AAA inflammation, expansion and rupture.

MMP inhibitors & AAAs

Tissue inhibitors of metalloproteinases (TIMPs) are natural inhibitors of MMPs, found in most tissues and body fluids. The balance between MMP and TIMP activity may affect both normal and pathological events – such as tissue remodeling, angiogenesis, invasion, tumor

genesis and metastasis [91]. An imbalance in the MMP: TIMP activity ratio may underlie the pathogenesis of vascular diseases, such as AAAs [92]. TIMP-1 plays a key role in preventing medial degradation through its ability to inhibit the MMPs involved in the disruption of the media [93]. In human AAAs, aortic tissue TIMP-1 (142.2 vs 302.8 ng/mg protein; $p = 0.01$) and TIMP-2 (9.2 vs 33.1 ng/mg protein; $p < 0.001$) levels were significantly lower than in normal aortas [73]. TIMP-2 is also reduced in the aortas of AAA and AOD patients, compared with that in control nondiseased aortas [94,95], supporting the hypothesis that MMPs are important proteases in experimental and human AAAs.

Functions of MMPs in experimental AAAs

Direct evidence for MMP participation in AAA formation came from experimental AAA using different MMP gene-targeted or transgenic animals (Table 2). Using MMP-9 and -12 gene-deficient mice, Pyo *et al.* showed that the absence of MMP-9, but not of macrophage elastase MMP-12, protected mice from aortic elastase perfusion-induced AAA formation. Aneurysmal degeneration was also inhibited by the MMP inhibitor doxycycline [96]. These lines of experiments proved a direct role of MMP-9, but not of MMP-12, in human AAAs, although both MMP-12 mRNA and protein levels were significantly higher in AAA lesions than in normal aortas [97]. In peri-aortic $CaCl_2$ injury-induced experimental AAAs, lesion formation was fully blocked in both MMP-9 and -2 gene-deficient mice. Reconstitution with macrophages from wild-type (WT) mice can restore AAA formation in MMP-9-deficient mice, but not in MMP-2-deficient mice, suggesting that MMP-9 from macrophages and MMP-2 from other cells (e.g., mesenchymal cells) contributed to the formation of these experimental AAAs [98]. In the same peri-aortic $CaCl_2$ injury-induced AAA formation in mice, transplantation with MT1-MMP-deficient bone marrow to WT littermates ablated AAA formation. Investigators demonstrated that macrophage-derived MT1-MMP played a dominant role in elastinolysis during AAA formation [99].

Genetic manipulation of TIMP expression helped to diagnose MMP functions further in AAA pathogenesis. TIMP-1-deficient *Apoe*$^{-/-}$ mice (*Timp1*$^{-/-}$ *Apoe*$^{-/-}$) that consumed a high-cholesterol diet for 30 weeks had reduced atherosclerosis, but increased aneurysms throughout the thoracic and abdominal aorta. Aortic tissue extracts contained increased MMP-2 and -9, as determined by gelatin gel zymography, but did not affect the activity of the serine proteases t-PA (tissue type plasminogen activator) and uPA (urokinase plasminogen activator), as determined by casein gel zymography assay [100]. In elastase perfusion-induced AAA in mice, aortic diameters 14 days postinfusion (1.62 ± 0.05 vs 1.33 ± 0.05 mm; $p < 0.001$) and total percentage of increase (208.4 ± 12.7 vs $153.6 \pm 9.5\%$; $p = 0.001$) were significantly larger in *Timp1*$^{-/-}$ mice than in WT mice. *Timp1*$^{-/-}$ mice showed a clear loss of aortic media elastin, but WT mice did not [101]. In intraluminal elastase perfusion-induced AAA in rats, local perfusion of adenovirus overexpressing TIMP-2 to the aortas completely prevented AAA formation, and preserved elastin and collagen fibers [102]. MMP-2-deficient mice, however, developed opposite phenotypes. In peri-aortic $CaCl_2$ injury-induced AAAs, aortic diameter increase was significantly suppressed in *Timp2*$^{-/-}$ mice compared with WT mice. Medial elastin fragmentation was not different between the two groups. Total and processed MMP-2 and -9 activities were not significantly different between the groups, although the investigators claimed reduction in active MMP-2 in *Timp2*$^{-/-}$ mouse aortas [103]. These observations suggest that TIMP-1 may be more important than TIMP-2 in regulating MMP activities during AAA formation, but whether the same is true in human AAA pathogenesis remains unknown. Table 2 summarizes these observations from different MMP and TIMP transgenic models of experimental AAAs.

MMP inhibitors in experimental AAAs

Observations from experimental AAA models confirmed an essential role of MMPs in AAA formation. The development of MMP inhibitors as therapeutic agents is important and necessary, but no MMP selective inhibitor has yet been tested in experimental AAAs or in AAA patients. Developing selective inhibitors to target individual MMPs is difficult, although one study has claimed that phosphinic peptide Br-Ph-(PO_2-CH_2)-X-Glu-Glu-NH_2 inhibited only MMP-12 [104]. Most (if not all) current MMP inhibitors target a broad spectrum of MMPs, and many have been tested in experimental AAA (Table 3) and in AAA patients (Table 4).

BB-94, also called batimastat, is a synthetic peptide backbone-based small molecule inhibitor of MMPs [105]. In elastase perfusion-induced AAAs in rats, daily intraperitoneal administration of BB-94 (15 mg/day) reduced aortic expansion from 157% in the untreated group to 115% in the treated group ($p = 0.026$), and also significantly reduced media elastin degradation and adventitia inflammatory cell infiltration [106]. The most widely used MMP inhibitor in AAAs is probably doxycycline – a tetracycline antibiotic that nonspecifically inhibits MMPs and affects MMP expression. In cultured human SMCs, doxycycline inhibited MMP-2 expression. In AAA explants, doxycycline also reduced MMP-2 and -9 expression (both active and latent forms) [107]. Several animal studies have demonstrated its beneficial effect in treating experimental AAAs. Among thioglycolate-plasmin aortic intraluminal perfusion-induced early AAAs in rats, daily subcutaneous injection of doxycycline significantly reduced aortic dilation, increased medial elastin contents and reduced lesion MMP-9 activities, compared with those treated with saline, at 7 days post-perfusion [108]. In elastase perfusion-induced AAAs in rats, doxycycline reduced AAA size in a dose-dependent manner, and reduced lesion MMP-9 activity and elastin destruction, but showed no effect on MMP-2 activity [109]. Similar effects were observed in Ang II infusion-induced AAAs in *Apoe*$^{-/-}$ mice. Doxycycline treatment (in drinking water) reduced AAA incidence from 86 to 35% [110]. Table 3 summarizes the activities of these MMP inhibitors in experimental AAAs.

MMP inhibitors in human patients

Successful suppression of AAAs in animals led to the development of doxycycline trials in humans. Several short-term and small-population trials demonstrated the beneficial role of doxycycline in human AAAs. A trial in Leiden, the Netherlands, used 13–15 AAA patients per group and treated them with different doses of doxycycline (50–300 mg/day) for 2 weeks. Doxycycline significantly suppressed AAA lesion MMP-8 and -9 protein levels, MMP-3 and -25 mRNA levels, lesion neutrophil and $CD8^+$ T-cell infiltration, and increased lesion TIMP-1 and cystatin C expression [111,112]. A randomized, placebo-controlled trial in St. Louis (MO, USA) used 20 EVAR patients who took 100 mg of doxycycline twice daily for 6 months. Doxycycline treatment significantly reduced plasma MMP-9 levels, maximum infrarenal aortic diameter and aortic neck diameter [113]. A prospective, double-blind, randomized, placebo-controlled study from Oulu, Finland, used 32 patients with an AAA diameter of <55 mm. Treatment with doxycycline at 150 mg/day for 3 months, followed by 6–18 months of surveillance, showed that doxycycline reduced AAA expansion rate ($p = 0.01$). At 6 months of follow-up, doxycycline significantly reduced plasma CRP levels compared with those from baseline ($p = 0.01$) [114]. However, some cohort studies showed conflicting results. A double-blind, randomized study in London, UK, demonstrated that doxycycline (100 mg once daily) treatment showed no differences from placebo in MMP-2, -3 and -9 activity, or expression of all eight MMPs tested (MMP-1, -2, -3, -7, -9, -11, -12 and -14) and TIMP-1 [115]. A prospective (Phase II) multicenter study of 36 AAA patients showed that AAA size did not change after 6 months of doxycycline treatment (100

mg orally, twice daily; before: 41.0 ± 0.9 mm; after: 42.7 ± 1.3 mm), although plasma MMP-9 levels were decreased at the 3- and 6-month time points, among which 21% of patients had increased 6-month plasma MMP-9 levels [116]. Whether doxycycline can be used to treat human AAAs is therefore inconclusive, likely due to the small number of patients, the lack of adjustment for confounding factors, the short-term drug exposure and the lack of long-term follow-up among all prior clinical studies (Table 4). Evidence for using doxycycline as an AAA drug in humans may be premature [117].

Other indirect MMP inhibitors in experimental AAAs

Several other molecules that can indirectly affect MMP activity or expression have also been tested in human and experimental AAAs. Trapidil, for example, is an anti-platelet agent and CD40–CD40 ligand pathway inhibitor. In cultured aortic tissues from AAA patients and AOD patients, trapidil or anti-CD40L antibody both inhibited mRNA and protein production of MMP-2, but not MMP-9 [118]. Common lipid-lowering statins have been widely tested in both human and experimental AAAs. In Ang II infusion-induced AAAs in *Apoe*$^{-/-}$ mice, simvastatin treatment (10 mg/kg/day, subcutaneous injection) reduced AAA size, lesion Mac-3^{+} macrophage content, MMP-2 and -9 activities, and neovascularization [119]. In elastase perfusion-induced AAAs in rats, daily gastric lavage administration of simvastatin reduced aneurysm diameter significantly, compared with placebo-treated rats (3.4 ± 0.08 vs 4.3 ± 0.19 mm; $p = 0.0001$). By an unknown mechanism, simvastatin reduced lesion MMP-9 levels, ECM expression and oxidative stress [120]. In elastase perfusion-induced AAAs in C57BL/6 mice and hypercholesterolemic *Apoe*$^{-/-}$ mice, simvastatin treatment significantly reduced AAA incidence and diameter, preserved medial SMC loss and elastin degradation, reduced AAA lesion MMP-9 expression, and increased lesion TIMP-1 expression [121]. These animal studies, as summarized in Table 3, suggested an application of these common human lipid-lowering drugs in human AAA therapy.

Statins as indirect MMP inhibitors in human patients

In human AAA organ culture studies, several statins exhibit MMP inhibitory activities. Cerivastatin significantly and dose-dependently inhibited the expression of total and active MMP-9 proteins in cultured human AAA lesions [122]. Aortic explants from AAA patients treated with pravastatin showed no effect on MMP-9 expression, but had increased expression of TIMP-1 and arterial cell apoptotic 22-kDa BAX [123]. Unlike in experimental AAAs, however, statin treatment yielded conflicting observations in AAA patients. A study from Iowa City (IA, USA) examined 211 patients with AAAs greater than 3 cm with at least 1 year of follow-up. The median linear AAA growth rate was significantly slower in statin users compared with the non-statin group (0.9 mm/year vs 2.9 mm/year; $p < 0.0001$) [124]. A much bigger study from Eindhoven, the Netherlands, enrolled 5892 AAA patients from the EUROSTAR registry, among which 731 patients received post-EVAR statin treatment. Compared with those without statin treatment, statin users had a significantly higher cumulative survival rate after 5 years of follow-up (81 vs 77%; $p = 0.005$) [125]. After adjustment for common AAA risk factors, statin use remained a predictor of improved survival ($p = 0.03$). Several similar studies, however, showed negative results of statin treatment among AAA patients. A study from Malmö, Sweden, examined 325 AAA patients treated with ($n = 127$) or without ($n = 198$) statin. As expected, statin users showed lower plasma MMP-9 ($p = 0.038$) and total cholesterol ($p < 0.0001$) levels than those from the non-statin groups, but statin users had no differences from non-statin users in AAA diameters (51 ± 13 vs 53 ± 16 mm; $p = 0.978$) [126]. Another study from five vascular centers in Australia and New Zealand examined statin effect among 652 patients with small AAAs (infrarenal aortic diameters 30–50 mm), whose diameters were monitored for a median of 5 years. Of 652 patients, 349 patients received treatment with different statins

(simvastatin [47%], atorvastatin [35%], pravastatin [17%] or fluvastatin [1%]). Although statin treatment did lower serum CRP levels – as in trials of patients with diabetes, rheumatoid arthritis, dyslipidemia or other cardiovascular complications [127–130] – it showed no effect on serum MMP-9 or IL-6 levels [131]. Before and after adjustment for AAA risk factors, patients with or without statin treatment showed no differences in AAA growth rate. Similar observations were obtained from all five centers if results were analyzed individually [131]. A study from Chichester, UK, confirmed the observations from Australia and New Zealand. Of 1231 patients with small AAAs (aortic diameter smaller than 5.5 cm), 383 patients received statin treatment. After a median of 3.2 years of follow-up, statin use did not associate with AAA growth rate ($p = 0.122$) [132]. All these studies used existing databases and had no detailed information on how long patients had been treated with statins, or exactly which statins were used (Table 4). Several smaller studies therefore were designed to test the effect of several statins on human AAAs. Evans *et al.* from Leicester, UK, enrolled 32 AAA patients, among which ten patients received 40 mg/day of simvastatin for 3 weeks before surgical repair. Although there is no information whether simvastatin affected AAA size or growth rate, it reduced MMP-9 levels in AAA aortic biopsies, as determined by zymography [133]. A separate study from the same group tested the effect of statin treatment in 17 asymptomatic AAA patients who received simvastatin, atorvastatin or pravastatin before open surgical repair. They obtained the same results – statin treatment reduced MMP-3 ($p = 0.009$) and MMP-9 ($p < 0.001$) protein levels significantly and increased TIMP-1 expression ($p = 0.033$) in the infrarenal aortic biopsies from those 17 patients, as determined by tissue extract ELISA, compared with those from 46 AAA patients without statin treatment, although this study did not provide information on how long these patients had used statins [134]. A study from Montreal, Canada, used aortic tissue extract immunoblot and zymography assays to show that aortic tissue from 19 AAA patients who received atorvastatin treatment for 6 months or more had reduced TGF-β signaling and MMP-13 expression, but that this statin had no significant effect on the expression or activities of MMP-1, -2, -3, -8 and -9, or those of TIMP-1, -2, -3 and -4. Patients receiving atorvastatin treatment (5.5 ± 0.4 cm) had similar AAA sizes to those from the non-atorvastatin group (5.9 ± 0.6 cm) [135]. This negative effect of atorvastatin was further confirmed by a double-blind, randomized controlled trial in Hull, UK, that involved 40 patients undergoing open surgical repair who received atorvastatin (80 mg) and placebo for 4 weeks. There were no significant differences in MMP-2, -8 and -9, TIMP-1 or TIMP-2 expression in aortic specimens and AAA wall stress between the atorvastatin group and the placebo group [136]. Atorvastatin did not affect patient baseline demographics, comorbidities or operative statistics. Therefore, although data from experimental AAAs in animals are favorable, neither doxycycline trials nor statin trials have yielded satisfactory results to suggest that MMP inhibition is beneficial to humans with AAAs.

Conclusion & future perspective

Cysteine protease cathepsins and MMPs certainly play important roles in the pathogenesis of AAAs. *In vitro* cell culture experiments and *in vivo* animal disease models have confirmed their direct participation in AAA formation. The main question, however, is whether human AAA formation is different from experimental AAAs. Although several experimental AAA models are widely used both in large and small animals, none fully recapitulate what occurs in human AAAs. The development of cathepsin-selective and MMP selective inhibitors is another important area. Besides their pathogenic roles, many of these cathepsins and MMPs have physiological significance in humans and animals. Selective inhibition of particular proteases without inhibiting other members of the same protease family becomes essential.

Compared with cysteinyl cathepsins, more research groups are studying MMPs. As discussed, MMP inhibitors have been tested in humans. Inconclusive results suggest that either MMP expression and activity are not causative factors of human AAAs, or that selective inhibition of particular MMPs is essential. Clinical dissatisfaction with MMP inhibitors implies that cysteinyl cathepsins may be more important than MMPs in human AAA development. Unlike MMPs, most major cathepsins – including CatS, CatK and CatL – have selective inhibitors [10]. Indeed, a few CatK inhibitors are already in clinical trials for various human diseases [10]. We expect that CatS- and CatL-selective inhibitors will soon become available and enter human trials. These cathepsin inhibitors may yield much better results in AAA patients – a hypothesis that requires more preclinical investigation and proof.

Acknowledgments

The authors thank S Karwacki for her editorial assistance.

References

1. Moll FL, Powell JT, Fraedrich G, et al. Management of abdominal aortic aneurysms clinical practice guidelines of the European society for vascular surgery. Eur J Vasc Endovasc Surg. 2011; 41(Suppl 1):S1–S58. [PubMed: 21215940]
2. Kochanek KD, Xu J, Murphy SL, Miniño AM, Kung HC. Deaths: final data for 2009. Natl Vital Stat Rep. 2011; 60(3):1–166. [PubMed: 22670489]
3. Svensjö S, Björck M, Gürtelschmid M, Djavani Gidlund K, Hellberg A, Wanhainen A. Low prevalence of abdominal aortic aneurysm among 65-year-old Swedish men indicates a change in the epidemiology of the disease. Circulation. 2011; 124(10):1118–1123. [PubMed: 21844079]
4. Wassef M, Upchurch GR Jr, Kuivaniemi H, Thompson RW, Tilson MD 3rd. Challenges and opportunities in abdominal aortic aneurysm research. J Vasc Surg. 2007; 45(1):192–198. [PubMed: 17210410]
5. Annambhotla S, Bourgeois S, Wang X, Lin PH, Yao Q, Chen C. Recent advances in molecular mechanisms of abdominal aortic aneurysm formation. World J Surg. 2008; 32(6):976–986. [PubMed: 18259804]
6. Daugherty A, Cassis LA. Mechanisms of abdominal aortic aneurysm formation. Curr Atheroscler Rep. 2002; 4(3):222–227. [PubMed: 11931720]
7. Nedeau AE, Pomposelli FB, Hamdan AD, et al. Endovascular vs open repair for ruptured abdominal aortic aneurysm. J Vasc Surg. 2012; 56(1):15–20. [PubMed: 22626871]
8. Nordon IM, Hinchliffe RJ, Loftus IM, Thompson MM. Pathophysiology and epidemiology of abdominal aortic aneurysms. Nat Rev Cardiol. 2011; 8(2):92–102. [PubMed: 21079638]
9. Cheng XW, Huang Z, Kuzuya M, Okumura K, Murohara T. Cysteine protease cathepsins in atherosclerosis-based vascular disease and its complications. Hypertension. 2011; 58(6):978–986. [PubMed: 21986502]
10. Qin Y, Shi GP. Cysteinyl cathepsins and mast cell proteases in the pathogenesis and therapeutics of cardiovascular diseases. Pharmacol Ther. 2011; 131(3):338–350. [PubMed: 21605595]
11. Abdul-Hussien H, Soekhoe RG, Weber E, et al. Collagen degradation in the abdominal aneurysm: a conspiracy of matrix metalloproteinase and cysteine collagenases. Am J Pathol. 2007; 170(3): 809–817. [PubMed: 17322367]
12. Chapman HA, Riese RJ, Shi GP. Emerging roles for cysteine proteases in human biology. Annu Rev Physiol. 1997; 59:63–88. [PubMed: 9074757]
13. Isenburg JC, Simionescu DT, Starcher BC, Vyavahare NR. Elastin stabilization for treatment of abdominal aortic aneurysms. Circulation. 2007; 115(13):1729–1737. [PubMed: 17372168]
14. Choke E, Cockerill GW, Dawson J, et al. Increased angiogenesis at the site of abdominal aortic aneurysm rupture. Ann NY Acad Sci. 2006; 1085:315–319. [PubMed: 17182949]

15. Choke E, Thompson MM, Dawson J, et al. Abdominal aortic aneurysm rupture is associated with increased medial neovascularization and overexpression of proangiogenic cytokines. Arterioscler Thromb Vasc Biol. 2006; 26(9):2077–2082. [PubMed: 16809548]
16. Sun J, Sukhova GK, Zhang J, et al. Cathepsin L activity is essential to elastase perfusion-induced abdominal aortic aneurysms in mice. Arterioscler Thromb Vasc Biol. 2011; 31(11):2500–2508. [PubMed: 21868704]
17. Shi GP, Sukhova GK, Kuzuya M, et al. Deficiency of the cysteine protease cathepsin S impairs microvessel growth. Circ Res. 2003; 92(5):493–500. [PubMed: 12600886]
18. Wang B, Sun J, Kitamoto S, et al. Cathepsin S controls angiogenesis and tumor growth via matrix-derived angiogenic factors. J Biol Chem. 281(9):6020–6029. [PubMed: 16365041]
19. Shi GP, Dolganov GM. Comprehensive transcriptome of proteases and protease inhibitors in vascular cells. Stroke. 2006; 37(2):537–541. [PubMed: 16373647]
20. Turk V, Turk B, Turk D. Lysosomal cysteine proteases: facts and opportunities. EMBO J. 2001; 20(17):4629–4633. [PubMed: 11532926]
21. Turk D, Guncar G. Lysosomal cysteine proteases (cathepsins): promising drug targets. Acta Crystallogr D Biol Crystallogr. 2003; 59(Pt 2):203–213. [PubMed: 12554931]
22. Conus S, Simon HU. Cathepsins: key modulators of cell death and inflammatory responses. Biochem Pharmacol. 2008; 76(11):1374–1382. [PubMed: 18762176]
23. Brix K, Dunkhorst A, Mayer K, Jordans S. Cysteine cathepsins: cellular roadmap to different functions. Biochimie. 2008; 90(2):194–207. [PubMed: 17825974]
24. Kaakinen R, Lindstedt KA, Sneck M, Kovanen PT, Oörni K. Angiotensin II increases expression and secretion of cathepsin F in cultured human monocyte-derived macrophages: an angiotensin II type 2 receptor-mediated effect. Atherosclerosis. 2007; 192(2):323–327. [PubMed: 16963053]
25. Yamashima T. $Ca2^{+}$-dependent proteases in ischemic neuronal death: a conserved 'calpain-cathepsin cascade' from nematodes to primates. Cell Calcium. 2004; 36(3–4):285–293. [PubMed: 15261484]
26. Rudensky A, Beers C. Lysosomal cysteine proteases and antigen presentation. Ernst Schering Res Found Workshop. 2006; (56):81–95. [PubMed: 16329647]
27. 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(3):576–583. [PubMed: 9691094]
28. Obermajer N, Jevnikar Z, Doljak B, Kos J. Role of cysteine cathepsins in matrix degradation and cell signalling. Connect Tissue Res. 2008; 49(3):193–196. [PubMed: 18661341]
29. Haendeler J, Popp R, Goy C, Tischler V, Zeiher AM, Dimmeler S. Cathepsin D and H2O2 stimulate degradation of thioredoxin-1: implication for endothelial cell apoptosis. J Biol Chem. 2005; 280(52):42945–42951. [PubMed: 16263712]
30. Boonen S, Rosenberg E, Claessens F, Vanderschueren D, Papapoulos S. Inhibition of cathepsin K for treatment of osteoporosis. Curr Osteoporos Rep. 2012; 10(1):73–79. [PubMed: 22228398]
31. Zhang ZB, Li ZG. Cathepsin B and phospo-JNK in relation to ongoing apoptosis after transient focal cerebral ischemia in the rat. Neurochem Res. 2012; 37(5):948–957. [PubMed: 22270907]
32. Stoeckle C, Quecke P, Rückrich T, et al. Cathepsin S dominates autoantigen processing in human thymic dendritic cells. J Autoimmun. 2012; 38(4):332–343. [PubMed: 22424724]
33. Sukhova GK, Shi GP. Do cathepsins play a role in abdominal aortic aneurysm pathogenesis? Ann NY Acad Sci. 2006; 1085:161–169. [PubMed: 17182932]
34. Liu J, Ma L, Yang J, et al. Increased serum cathepsin S in patients with atherosclerosis and diabetes. Atherosclerosis. 2006; 186(2):411–419. [PubMed: 16140306]
35. Pan L, Li Y, Jia L, et al. Cathepsin S deficiency results in abnormal accumulation of autophagosomes in macrophages and enhances ang II-induced cardiac inflammation. PLoS One. 2012; 7(4):e35315. [PubMed: 22558139]
36. Lee YT, Chen SC, Shyu LY, et al. Significant elevation of plasma cathepsin B and cystatin C in patients with community-acquired pneumonia. Clin Chim Acta. 2012; 413(5–6):630–635. [PubMed: 22209964]

37. Boonen S, Rosenberg E, Claessens F, Vanderschueren D, Papapoulos S. Inhibition of cathepsin K for treatment of osteoporosis. Curr Osteoporos Rep. 2012; 10(1):73–79. [PubMed: 22228398]
38. Kim N, Bae KB, Kim MO, et al. Overexpression of cathepsin S induces chronic atopic dermatitis in mice. J Invest Dermatol. 2012; 132(4):1169–1176. [PubMed: 22170489]
39. Wu D, Wang H, Li Z, et al. Cathepsin B may be a potential biomarker in cervical cancer. Histol Histopathol. 2012; 27(1):79–87. [PubMed: 22127599]
40. Kindy MS, Yu J, Zhu H, El-Amouri SS, Hook V, Hook GR. Deletion of the cathepsin B gene improves memory deficits in a transgenic Alzheimer's disease mouse model expressing AβPP containing the wild-type β-secretase site sequence. J Alzheimers Dis. 2012; 29(4):827–840. [PubMed: 22337825]
41. Samokhin AO, Lythgo PA, Gauthier JY, Percival MD, Brömme D. Pharmacological inhibition of cathepsin S decreases atherosclerotic lesions in *Apoe*$^{-/-}$ mice. J Cardiovasc Pharmacol. 2010; 56(1):98–105. [PubMed: 20410833]
42. Lutgens SP, Cleutjens KB, Daemen MJ, Heeneman S. Cathepsin cysteine proteases in cardiovascular disease. FASEB J. 2007; 21(12):3029–3041. [PubMed: 17522380]
43. Keegan PM, Wilder CL, Platt MO. Tumor necrosis factor α stimulates cathepsin K and V activity via juxtacrine monocyte-endothelial cell signaling and JNK activation. Mol Cell Biochem. 2012 (In Press).
44. Liu J, Sukhova GK, Sun JS, Xu WH, Libby P, Shi GP. Lysosomal cysteine proteases in atherosclerosis. Arterioscler Thromb Vasc Biol. 2004; 24(8):1359–1366. [PubMed: 15178558]
45. Lv BJ, Lindholt JS, Cheng X, Wang J, Shi GP. Plasma cathepsin s and cystatin C levels and risk of abdominal aortic aneurysm: a randomized population-based study. PLoS One. 2012; 7(7):e41813. [PubMed: 22844527]
46. Qin Y, Cao X, Guo J, et al. Deficiency of cathepsin S attenuates angiotensin II-induced abdominal aortic aneurysm formation in apolipoprotein E-deficient mice. Cardiovasc Res. 2012 (In Press).
47. Sun J, Sukhova GK, Zhang J, et al. Cathepsin K deficiency reduces elastase perfusion-induced abdominal aortic aneurysms in mice. Arterioscler Thromb Vasc Biol. 2012; 32(1):15–23. [PubMed: 21817099]
48. Bai L, Beckers L, Wijnands E, et al. Cathepsin K gene disruption does not affect murine aneurysm formation. Atherosclerosis. 2010; 209(1):96–103. [PubMed: 19775691]
49. Liu J, Sukhova GK, Yang JT, et al. Cathepsin L expression and regulation in human abdominal aortic aneurysm, atherosclerosis, and vascular cells. Atherosclerosis. 2006; 184(2):302–311. [PubMed: 15982660]
50. Shi GP. Role of cathepsin C in elastase-induced mouse abdominal aortic aneurysms. Future Cardiol. 2007; 3(6):591–593. [PubMed: 19804279]
51. Gacko M, Guzowski A, Wożniak A, Worowska A, Greczaniuk A. Concentration and activity of cathepsin D in the blood plasma and serum of patients with abdominal aortic aneurysm. Przegl Lek. 2006; 63(5):265–267. [PubMed: 17036502]
52. Siergiejuk M, Gacko M, Worowska A. Cathepsin A activity of a parietal thrombus of an abdominal aortic aneurysm. Folia Histochem Cytobiol. 2011; 49(1):10–12. [PubMed: 21526484]
53. Shi GP, Sukhova GK, Grubb A, et al. Cystatin C deficiency in human atherosclerosis and aortic aneurysms. J Clin Invest. 1999; 104(9):1191–1197. [PubMed: 10545518]
54. Lindholt JS, Erlandsen EJ, Henneberg EW. Cystatin C deficiency is associated with the progression of small abdominal aortic aneurysms. Br J Surg. 2001; 88(11):1472–1475. [PubMed: 11683743]
55. Sukhova GK, Wang B, Libby P, et al. Cystatin C deficiency increases elastic lamina degradation and aortic dilatation in apolipoprotein E-null mice. Circ Res. 2005; 96(3):368–375. [PubMed: 15653570]
56. Schulte S, Sun J, Libby P, et al. Cystatin C deficiency promotes inflammation in angiotensin II-induced abdominal aortic aneurisms in atherosclerotic mice. Am J Pathol. 2010; 177(1):456–463. [PubMed: 20472891]
57. Arnlöv J. Cathepsin S as a biomarker: where are we now and what are the future challenges? Biomark Med. 2012; 6(1):9–11. [PubMed: 22296192]

58. Jobs E, Ingelsson E, Risérus U, et al. Association between serum cathepsin S and mortality in older adults. JAMA. 2011; 306(10):1113–1121. [PubMed: 21878432]
59. Fu X, Parks WC, Heinecke JW. Activation and silencing of matrix metalloproteinases. Semin Cell Dev Biol. 2008; 19(1):2–13. [PubMed: 17689277]
60. Van Lint P, Libert C. Chemokine and cytokine processing by matrix metalloproteinases and its effect on leukocyte migration and inflammation. J Leukoc Biol. 2007; 82(6):1375–1381. [PubMed: 17709402]
61. Wadsworth SJ, Atsuta R, McIntyre JO, Hackett TL, Singhera GK, Dorscheid DR. IL-13 and TH2 cytokine exposure triggers matrix metalloproteinase 7-mediated Fas ligand cleavage from bronchial epithelial cells. J Allergy Clin Immunol. 2010; 126(2):366–374. [PubMed: 20624652]
62. Wang M, Zhao D, Spinetti G, et al. Matrix metalloproteinase 2 activation of transforming growth factor-β1 (TGF-β1) and TGF-β1-type II receptor signaling within the aged arterial wall. Arterioscler Thromb Vasc Biol. 2006; 26(7):1503–1539. [PubMed: 16690877]
63. Mu D, Cambier S, Fjellbirkeland L, et al. The integrin α(v) β8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-β1. J Cell Biol. 2002; 157(3):493–507. [PubMed: 11970960]
64. Makowski GS, Ramsby ML. Autoactivation profiles of calcium-dependent matrix metalloproteinase-2 and -9 in inflammatory synovial fluid: effect of pyrophosphate and bisphosphonates. Clin Chim Acta. 2005; 358(1–2):182–191. [PubMed: 15921672]
65. Vessillier S, Adams G, Chernajovsky Y. Latent cytokines: development of novel cleavage sites and kinetic analysis of their differential sensitivity to MMP-1 and MMP-3. Protein Eng Des Sel. 2004; 17(12):829–835. [PubMed: 15708865]
66. Zahradka P, Harding G, Litchie B, et al. Activation of MMP-2 in response to vascular injury is mediated by phosphatidylinositol 3-kinase-dependent expression of MT1-MMP. Am J Physiol Heart Circ Physiol. 2004; 287(6):H2861–H2870. [PubMed: 15297252]
67. Eagleton MJ, Ballard N, Lynch E, Srivastava SD, Upchurch GR Jr, Stanley JC. Early increased MT1-MMP expression and late MMP-2 and MMP-9 activity during angiotensin II induced aneurysm formation. J Surg Res. 2006; 135(2):345–351. [PubMed: 16716358]
68. Newman KM, Jean-Claude J, Li H, et al. Cellular localization of matrix metalloproteinases in the abdominal aortic aneurysm wall. J Vasc Surg. 1994; 20(5):814–820. [PubMed: 7526009]
69. Fontaine V, Jacob MP, Houard X, et al. Involvement of the mural thrombus as a site of protease release and activation in human aortic aneurysms. Am J Pathol. 2002; 161(5):1701–1710. [PubMed: 12414517]
70. Nollendorfs A, Greiner TC, Nagase H, Baxter BT. The expression and localization of membrane type-1 matrix metalloproteinase in human abdominal aortic aneurysms. J Vasc Surg. 2001; 34(2): 316–322. [PubMed: 11496285]
71. Davis V, Persidskaia R, Baca-Regen L, et al. Matrix metalloproteinase-2 production and its binding to the matrix are increased in abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol. 1998; 18(10):1625–1633. [PubMed: 9763536]
72. Koenig GC, Rowe RG, Day SM, et al. MT1-MMP-dependent remodeling of cardiac extracellular matrix structure and function following myocardial infarction. Am J Pathol. 2012; 180(5):1863–1878. [PubMed: 22464947]
73. Wilson WR, Schwalbe EC, Jones JL, Bell PR, Thompson MM. Matrix metalloproteinase 8 (neutrophil collagenase) in the pathogenesis of abdominal aortic aneurysm. Br J Surg. 2005; 92(7): 828–833. [PubMed: 15918165]
74. Petersen E, Gineitis A, Wågberg F, Angquist KA. Activity of matrix metalloproteinase-2 and -9 in abdominal aortic aneurysms Relation to size and rupture. Eur J Vasc Endovasc Surg. 2000; 20(5): 457–461. [PubMed: 11112465]
75. Freestone T, Turner RJ, Coady A, Higman DJ, Greenhalgh RM, Powell JT. Inflammation and matrix metalloproteinases in the enlarging abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol. 1995; 15(8):1145–1151. [PubMed: 7627708]
76. McMillan WD, Pearce WH. Increased plasma levels of metalloproteinase-9 are associated with abdominal aortic aneurysms. J Vasc Surg. 1999; 29(1):122–127. [PubMed: 9882796]

77. Tromp G, Gatalica Z, Skunca M, et al. Elevated expression of matrix metalloproteinase-13 in abdominal aortic aneurysms. Ann Vasc Surg. 2004; 18(4):414–420. [PubMed: 15156361]

78. Rizas KD, Ippagunta N, Tilson MD 3rd. Immune cells and molecular mediators in the pathogenesis of the abdominal aortic aneurysm. Cardiol Rev. 2009; 17(5):201–210. [PubMed: 19690470]

79. Ahluwalia N, Lin AY, Tager AM, et al. Inhibited aortic aneurysm formation in BLT1-deficient mice. J Immunol. 2007; 179(1):691–697. [PubMed: 17579092]

80. Juvonen J, Surcel HM, Satta J, et al. Elevated circulating levels of inflammatory cytokines in patients with abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol. 1997; 17(11):2843–2847. [PubMed: 9409264]

81. Eliason JL, Hannawa KK, Ailawadi G, et al. Neutrophil depletion inhibits experimental abdominal aortic aneurysm formation. Circulation. 2005; 112(2):232–240. [PubMed: 16009808]

82. Sun J, Sukhova GK, Yang M, et al. Mast cells modulate the pathogenesis of elastase-induced abdominal aortic aneurysms in mice. J Clin Invest. 2007; 117(11):3359–3368. [PubMed: 17932568]

83. Eugster T, Huber A, Obeid T, Schwegler I, Gürke L, Stierli P. Aminoterminal propeptide of type III procollagen and matrix metalloproteinases-2 and -9 failed to serve as serum markers for abdominal aortic aneurysm. Eur J Vasc Endovasc Surg. 2005; 29(4):378–382. [PubMed: 15749038]

84. van Laake LW, Vainas T, Dammers R, Kitslaar PJ, Hoeks AP, Schurink GW. Systemic dilation diathesis in patients with abdominal aortic aneurysms: a role for matrix metalloproteinase-9? Eur J Vasc Endovasc Surg. 2005; 29(4):371–377. [PubMed: 15749037]

85. Watanabe T, Sato A, Sawai T, et al. The elevated level of circulating matrix metalloproteinase-9 in patients with abdominal aortic aneurysms decreased to levels equal to those of healthy controls after an aortic repair. Ann Vasc Surg. 2006; 20(3):317–321. [PubMed: 16779512]

86. Hovsepian DM, Ziporin SJ, Sakurai MK, Lee JK, Curci JA, Thompson RW. Elevated plasma levels of matrix metalloproteinase-9 in patients with abdominal aortic aneurysms: a circulating marker of degenerative aneurysm disease. J Vasc Interv Radiol. 2000; 11(10):1345–1352. [PubMed: 11099248]

87. Hellenthal FA, Ten Bosch JA, Pulinx B, et al. Plasma levels of matrix metalloproteinase-9: a possible diagnostic marker of successful endovascular aneurysm repair. Eur J Vasc Endovasc Surg. 2012; 43(2):171–172. [PubMed: 22172237]

88. Grodin JL, Powell-Wiley TM, Ayers CR, et al. Circulating levels of matrix metalloproteinase-9 and abdominal aortic pathology: from the Dallas Heart Study. Vasc Med. 2011; 16(5):339–345. [PubMed: 22002999]

89. Wilson WR, Anderton M, Choke EC, Dawson J, Loftus IM, Thompson MM. Elevated plasma MMP1 and MMP9 are associated with abdominal aortic aneurysm rupture. Eur J Vasc Endovasc Surg. 2008; 35(5):580–584. [PubMed: 18226564]

90. Wilson WR, Anderton M, Schwalbe EC, et al. Matrix metalloproteinase-8 and -9 are increased at the site of abdominal aortic aneurysm rupture. Circulation. 2006; 113(3):438–445. [PubMed: 16432074]

91. Lambert E, Dassé E, Haye B, Petitfrère E. TIMPs as multifacial proteins. Crit Rev Oncol Hematol. 2004; 49(3):187–198. [PubMed: 15036259]

92. Raffetto JD, Khalil RA. Matrix metalloproteinases and their inhibitors in vascular remodeling and vascular disease. Biochem Pharmacol. 2008; 75(2):346–359. [PubMed: 17678629]

93. Lemaître V, Soloway PD, D'Armiento J. Increased medial degradation with pseudo-aneurysm formation in apolipoprotein E-knockout mice deficient in tissue inhibitor of metalloproteinases-1. Circulation. 2003; 107(2):333–338. [PubMed: 12538437]

94. Defawe OD, Colige A, Lambert CA, et al. TIMP-2 and PAI-1 mRNA levels are lower in aneurysmal as compared to athero-occlusive abdominal aortas. Cardiovasc Res. 2003; 60(1):205–213. [PubMed: 14522424]

95. Antoniou GA, Tentes IK, Antoniou SA, et al. Circulating matrix metalloproteinases and their inhibitors in inguinal hernia and abdominal aortic aneurysm. Int Angiol. 2011; 30(2):123–129. [PubMed: 21427648]

96. Pyo R, Lee JK, Shipley JM, et al. Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. J Clin Invest. 2000; 105(11):1641–1649. [PubMed: 10841523]

97. Curci JA, Liao S, Huffman MD, Shapiro SD, Thompson RW. Expression and localization of macrophage elastase (matrix metalloproteinase-12) in abdominal aortic aneurysms. J Clin Invest. 1998; 102(11):1900–1910. [PubMed: 9835614]

98. Longo GM, Xiong W, Greiner TC, Zhao Y, Fiotti N, Baxter BT. Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. J Clin Invest. 2002; 110(5):625–632. [PubMed: 12208863]

99. Xiong W, Knispel R, MacTaggart J, Greiner TC, Weiss SJ, Baxter BT. Membrane-type 1 matrix metalloproteinase regulates macrophage-dependent elastolytic activity and aneurysm formation *in vivo*. J Biol Chem. 2009; 284(3):1765–1771. [PubMed: 19010778]

100. Silence J, Collen D, Lijnen HR. Reduced atherosclerotic plaque but enhanced aneurysm formation in mice with inactivation of the tissue inhibitor of metalloproteinase-1 (TIMP-1) gene. Circ Res. 2002; 90(8):897–903. [PubMed: 11988491]

101. Eskandari MK, Vijungco JD, Flores A, Borensztajn J, Shively V, Pearce WH. Enhanced abdominal aortic aneurysm in TIMP-1-deficient mice. J Surg Res. 2005; 123(2):289–293. [PubMed: 15680392]

102. Zhao X, Li H, Dong J, Kokudo N, Tang W. Overexpression of TIMP-2 mediated by recombinant adenovirus in rat abdominal aorta inhibits extracellular matrix degradation. Biosci Trends. 2008; 2(5):206–210. [PubMed: 20103929]

103. Xiong W, Knispel R, Mactaggart J, Baxter BT. Effects of tissue inhibitor of metalloproteinase 2 deficiency on aneurysm formation. J Vasc Surg. 2006; 44(5):1061–1066. [PubMed: 17098543]

104. Devel L, Rogakos V, David A, et al. Development of selective inhibitors and substrate of matrix metalloproteinase-12. J Biol Chem. 2006; 281(16):11152–11160. [PubMed: 16481329]

105. Davies B, Brown PD, East N, Crimmin MJ, Balkwill FR. A synthetic matrix metalloproteinase inhibitor decreases tumor burden and prolongs survival of mice bearing human ovarian carcinoma xenografts. Cancer Res. 1993; 53(9):2087–2091. [PubMed: 8347186]

106. Bigatel DA, Elmore JR, Carey DJ, Cizmeci-Smith G, Franklin DP, Youkey JR. The matrix metalloproteinase inhibitor BB-94 limits expansion of experimental abdominal aortic aneurysms. J Vasc Surg. 1999; 29(1):130–138. [PubMed: 9882797]

107. Liu J, Xiong W, Baca-Regen L, Nagase H, Baxter BT. Mechanism of inhibition of matrix metalloproteinase-2 expression by doxycycline in human aortic smooth muscle cells. J Vasc Surg. 2003; 38(6):1376–1383. [PubMed: 14681644]

108. Kaito K, Urayama H, Watanabe G. Doxycycline treatment in a model of early abdominal aortic aneurysm. Surg Today. 2003; 33(6):426–433. [PubMed: 12768368]

109. Curci JA, Petrinec D, Liao S, Golub LM, Thompson RW. Pharmacologic suppression of experimental abdominal aortic aneurysms: a comparison of doxycycline and four chemically modified tetracyclines. J Vasc Surg. 1998; 28(6):1082–1093. [PubMed: 9845660]

110. Manning MW, Cassis LA, Daugherty A. Differential effects of doxycycline, a broad-spectrum matrix metalloproteinase inhibitor, on angiotensin II-induced atherosclerosis and abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol. 2003; 23(3):483–488. [PubMed: 12615694]

111. Lindeman JH, Abdul-Hussien H, van Bockel JH, Wolterbeek R, Kleemann R. Clinical trial of doxycycline for matrix metalloproteinase-9 inhibition in patients with an abdominal aneurysm: doxycycline selectively depletes aortic wall neutrophils and cytotoxic T cells. Circulation. 2009; 119(16):2209–2216. [PubMed: 19364980]

112. Abdul-Hussien H, Hanemaaijer R, Verheijen JH, van Bockel JH, Geelkerken RH, Lindeman JH. Doxycycline therapy for abdominal aneurysm: improved proteolytic balance through reduced neutrophil content. J Vasc Surg. 2009; 49(3):741–749. [PubMed: 19268776]

113. Hackmann AE, Rubin BG, Sanchez LA, Geraghty PA, Thompson RW, Curci JA. A randomized, placebo-controlled trial of doxycycline after endoluminal aneurysm repair. J Vasc Surg. 2008; 48(3):519–526. [PubMed: 18632241]

114. Mosorin M, Juvonen J, Biancari F, et al. Use of doxycycline to decrease the growth rate of abdominal aortic aneurysms: a randomized, double-blind, placebo-controlled pilot study. J Vasc Surg. 2001; 34(4):606–610. [PubMed: 11668312]
115. Ding R, McGuinness CL, Burnand KG, Sullivan E, Smith A. Matrix metalloproteinases in the aneurysm wall of patients treated with low-dose doxycycline. Vascular. 2005; 13(5):290–297. [PubMed: 16288704]
116. Baxter BT, Pearce WH, Waltke EA, et al. Prolonged administration of doxycycline in patients with small asymptomatic abdominal aortic aneurysms: report of a prospective (Phase II) multicenter study. J Vasc Surg. 2002; 36(1):1–12. [PubMed: 12096249]
117. Dodd BR, Spence RA. Doxycycline inhibition of abdominal aortic aneurysm growth: a systematic review of the literature. Curr Vasc Pharmacol. 2011; 9(4):471–478. [PubMed: 21595625]
118. Nagashima H, Aoka Y, Sakomura Y, et al. Matrix metalloproteinase 2 is suppressed by trapidil, a CD40-CD40 ligand pathway inhibitor, in human abdominal aortic aneurysm wall. J Vasc Surg. 2004; 39(2):447–453. [PubMed: 14743151]
119. Zhang Y, Naggar JC, Welzig CM, et al. Simvastatin inhibits angiotensin II-induced abdominal aortic aneurysm formation in apolipoprotein E-knockout mice: possible role of ERK. Arterioscler Thromb Vasc Biol. 2009; 29(11):1764–1771. [PubMed: 19729613]
120. Kalyanasundaram A, Elmore JR, Manazer JR, et al. Simvastatin suppresses experimental aortic aneurysm expansion. J Vasc Surg. 2006; 43(1):117–124. [PubMed: 16414398]
121. Steinmetz EF, Buckley C, Shames ML, et al. Treatment with simvastatin suppresses the development of experimental abdominal aortic aneurysms in normal and hypercholesterolemic mice. Ann Surg. 2005; 241(1):92–101. [PubMed: 15621996]
122. Nagashima H, Aoka Y, Sakomura Y, et al. A 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, cerivastatin, suppresses production of matrix metalloproteinase-9 in human abdominal aortic aneurysm wall. J Vasc Surg. 2002; 36(1):158–163. [PubMed: 12096274]
123. Mateos-Cáceres PJ, López-Farré AJ, Morata PC, et al. Pravastatin increases the expression of the tissue inhibitor of matrix metalloproteinase-1 and the oncogene Bax in human aortic abdominal aneurysms. Can J Physiol Pharmacol. 2008; 86(7):431–437. [PubMed: 18641692]
124. Karrowni W, Dughman S, Hajj GP, Miller FJ Jr. Statin therapy reduces growth of abdominal aortic aneurysms. J Investig Med. 2011; 59(8):1239–1243.
125. Leurs LJ, Visser P, Laheij RJ, Buth J, Harris PL, Blankensteijn JD. Statin use is associated with reduced all-cause mortality after endovascular abdominal aortic aneurysm repair. Vascular. 2006; 14(1):1–8. [PubMed: 16849016]
126. Gottsäter A, Flondell-Site D, Kölbel T, Lindblad B. Associations between statin treatment and markers of inflammation, vasoconstriction, and coagulation in patients with abdominal aortic aneurysm. Vasc Endovasc Surg. 2008; 42(6):567–573.
127. Muhlestein JB, May HT, Jensen JR, et al. The reduction of inflammatory biomarkers by statin, fibrate, and combination therapy among diabetic patients with mixed dyslipidemia: the DIACOR (Diabetes and Combined Lipid Therapy Regimen) study. J Am Coll Cardiol. 2006; 48(2):396–401. [PubMed: 16843192]
128. Mäki-Petäjä KM, Booth AD, Hall FC, et al. Ezetimibe and simvastatin reduce inflammation, disease activity, and aortic stiffness and improve endothelial function in rheumatoid arthritis. J Am Coll Cardiol. 2007; 50(9):852–858. [PubMed: 17719471]
129. Li JJ, Wang Y, Nie SP, et al. Reduction of C-reactive protein by a single 80 mg of simvastatin in patients with unstable angina. Clin Chim Acta. 2007; 376(1–2):163–167. [PubMed: 16999946]
130. Wiviott SD, de Lemos JA, Cannon CP, et al. A tale of two trials: a comparison of the post-acute coronary syndrome lipid-lowering trials A to Z and PROVE IT-TIMI 22. Circulation. 2006; 113(11):1406–1414. [PubMed: 16534008]
131. Ferguson CD, Clancy P, Bourke B, et al. Association of statin prescription with small abdominal aortic aneurysm progression. Am Heart J. 2010; 159(2):307–313. [PubMed: 20152231]
132. Thompson AR, Cooper JA, Ashton HA, Hafez H. Growth rates of small abdominal aortic aneurysms correlate with clinical events. Br J Surg. 2010; 97(1):37–44. [PubMed: 20013940]

133. Evans J, Powell JT, Schwalbe E, Loftus IM, Thompson MM. Simvastatin attenuates the activity of matrix metalloprotease-9 in aneurysmal aortic tissue. Eur J Vasc Endovasc Surg. 2007; 34(3): 302–303. [PubMed: 17574455]

134. Wilson WR, Evans J, Bell PR, Thompson MM. HMG-CoA reductase inhibitors (statins) decrease MMP-3 and MMP-9 concentrations in abdominal aortic aneurysms. Eur J Vasc Endovasc Surg. 2005; 30(3):259–262. [PubMed: 16009575]

135. Schweitzer M, Mitmaker B, Obrand D, et al. Atorvastatin modulates matrix metalloproteinase expression, activity, and signaling in abdominal aortic aneurysms. Vasc Endovasc Surg. 2010; 44(2):116–122.

136. Rahman MN, Khan JA, Mazari FA, Mockford K, McCollum PT, Chetter IC. A randomized placebo controlled trial of the effect of preoperative statin use on matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in areas of low and peak wall stress in patients undergoing elective open repair of abdominal aortic aneurysm. Ann Vasc Surg. 2011; 25(1):32–38. [PubMed: 20889295]

Website

201. Pearce, WH. Abdominal aortic aneurysm. http://emedicine.medscape.com/article/463354

Executive summary

Cysteinyl cathepsins in abdominal aortic aneurysms

- Human and animal abdominal aortic aneurysm (AAA) lesions have increased expression of cathepsins S, K, L and C, which localize to lesion inflammatory cells and vascular cells.
- Plasma CatS levels are significantly higher in AAA patients than in age-matched healthy controls.
- Mice deficient in these cathepsins are protected from experimental AAA formation.

Matrix metalloproteinases in AAAs

- MMP-2, -3, -8, -9, -13 and MT-1 MMP are potent collagenases or elastases, and their expression and activities increase in human or animal AAA lesions or in the blood.
- Experimental AAAs revealed that MMP-9 from inflammatory cells and MMP-2 from mesenchymal cells are essential to AAA pathogenesis.

Endogenous cathepsin & MMP inhibitors in AAAs

- Cystatin C protein levels are reduced or deficient in human AAA lesions or plasma samples.
- Cystatin C deficiency increased aortic tissue cathepsin activity and enlarged aorta size in *Apoe*$^{-/-}$ mice, with or without Ang II infusion.
- TIMP-1 and -2 are often reduced in human AAA lesions. Deficiency of these MMP inhibitors increases the size of experimental AAAs.

Cathepsin & MMP inhibitors in AAA therapy

- Although selective inhibitors for several major AAA cathepsins are available, none have been tested in experimental AAAs or in humans.
- Nonselective MMP inhibitors (BB-94 and doxycycline) suppressed experimental AAA formation, but their application in humans remains uncertain.

Conclusion

- Increased expression of cathepsins and MMPs, but reduced levels of their inhibitors, in human AAA lesions suggest a role of these proteases in AAAs.
- Reduced AAA size and incidence in cathepsin-deficient and MMP-deficient mice, but enlarged aortic size in the absence of their inhibitors, proves that these proteases participate directly in AAA pathogenesis.
- No inhibitor, however, has successfully and consistently suppressed human AAAs in clinical trials.

Table 1

Cysteinyl cathepsins in different pathological events from experimental abdominal aortic aneurysms.

Pathological event	Cathepsins
Lesion leukocyte recruitment (e.g., macrophage, T cell and neutrophil)	CatS, CatL, CatK, CatC[†]
Lesion inflammatory cell proliferation (e.g., T cells)	CatS, CatL, CatK
EC and SMC cathepsin and MMP expression or activity	CatL, CatK
Lesion CatK and MMP-2/9 expression and activity	CatS
Lesion CatS/K activity and expression	CatL, CatK
Lesion elastin and collagen degradation	CatS, CatL, CatK
Lesion SMC apoptosis and loss	CatS, CatK (not CatL)
Other lesion cell apoptosis	CatS, CatK (not CatL)
Angiogenesis	CatS, CatL (not CatK)

[†]CatC activities on other listed events were not tested.

EC: Endothelial cell; MMP: Matrix metalloproteinase; SMC: Smooth-muscle cell.

Table 2

Role of matrix metalloproteinases and tissue inhibitors of metalloproteinases in different experimental abdominal aortic aneurysm models.

Proteases/inhibitors	AAA models	Animals	Observations	Ref.
Mmp2$^{-/-}$	Peri-aortic $CaCl_2$ injury	Mice	Reduced AAA	[98]
Mmp9$^{-/-}$	Peri-aortic $CaCl_2$ injury	Mice	Reduced AAA	[98]
Mmp9$^{-/-}$	Elastase perfusion	Mice	Reduced AAA	[96]
Mmp12$^{-/-}$	Elastase perfusion	Mice	No effect †	[96]
MT1-MMP$^{-/-}$	Bone marrow transplantation followed by $CaCl_2$ injury	Mice	Reduced AAA	[99]
Timp1$^{-/-}$*Apoe*$^{-/-}$	High-cholesterol diet	Mice	Increased AAA	[100]
Timp1$^{-/-}$	Elastase perfusion	Mice	Increased AAA	[101]
TIMP-2	Local overexpression followed by elastase perfusion	Rat	Reduced AAA	[102]
Timp2$^{-/-}$	Peri-aortic $CaCl_2$ injury	Mice	Reduced AAA †	[103]

† Study with unexpected results.

AAA: Abdominal aortic aneurysm.

Table 3

Direct and indirect matrix metalloproteinase inhibitors in reducing experimental abdominal aortic aneurysms.

Inhibitors	AAA models	Animals	Additional observations	Ref.
BB-94 (batimastat)	Elastase perfusion	Rats	Reduced lesion inflammation and increased elastin preservation	[106]
Doxycycline	Elastase perfusion	Mice	–	[96]
Doxycycline	Thioglycolate-plasmin perfusion	Rats	Reduced lesion MMP-9 activity and elastin degradation, no effect on MMP-2 activity	[108]
Doxycycline	Elastase perfusion	Rats	Reduced lesion MMP-9 activity and elastin degradation, no effect on MMP-2 activity	[109]
Doxycycline	Ang II infusion	Mice	No effect on systolic pressure or serum lipid profiles	[110]
Simvastatin	Ang II infusion	Mice	Reduced lesion MMP-2 and -9 activities, inflammation and neovascularization	[119]
Simvastatin	Elastase perfusion	Rats	Reduced lesion MMP-9 levels, ECM expression and oxidative stress	[120]
Simvastatin	Elastase perfusion	Mice	Reduced lesion MMP-9, increased lesion TIMP-1 expression, and reduced media SMC loss and elastin degradation	[121]

AAA: Abdominal aortic aneurysm; ECM: Extracellular matrix; SMC: Smooth-muscle cell.

Table 4

Direct and indirect matrix metalloproteinase inhibitors in abdominal aortic aneurysm clinical trials.

Inhibitors	Population and treatment duration[†]	Results	Ref.
Doxycycline	Leiden, the Netherlands (n = 13–15/60), 2 weeks	Reduced lesion MMP expression and activity, reduced lesion T cells and neutrophils, and increased lesion cystatin C and TIMP-1 expression	[111,112]
Doxycycline	St. Louis, MO, USA (n = 20/44), 6 months	Reduced plasma MMP-9 and reduced AAA diameter	[113]
Doxycycline	Olulu and Kajaani, Finland (n = 17/32), 3 months	Reduced AAA expansion rate and plasma CRP	[114]
Doxycycline[‡]	London, UK (n = 23/56), 1 month	No effect on MMP or TIMP-1 expression or activity	[115]
Doxycycline[‡]	Multicenter (n = 36), 6 months	No effect on AAA or expansion rate; some patients had increased plasma MMP-9	[116]
Statins[§]	Iowa City, IA, USA (n = 136/211), no record of duration	Reduced AAA growth rate	[124]
Statins[§]	Eindhoven, the Netherlands (n = 731/5892), no record of duration	Improved post-endovascular surgical repair survival	[125]
Statins[§]	Malmö, Sweden (n = 127/325), no record of duration	Reduced plasma MMP-9 and cholesterol, no effect on plasma MMP-2 and AAA diameters	[126]
Statins[§‡]	Multicenter (n = 349/652), no record of duration	Reduced serum CRP and total cholesterol, no effect on plasma MMP or IL-6 and no effect on AAA growth	[131]
Statins[§‡]	Chichester, UK (n = 383/1231), no record of duration	No association with AAA growth rate	[132]
Simvastatin	Leicester, UK (n = 10/21), 3 weeks	Reduced lesion MMP-9 expression	[133]
Mixed statins[¶]	Leicester, UK (n = 17/63), no record of duration	Reduced lesion MMP-3 and -9 expression, increased TIMP-1 expression, no effect on AAA size	[134]
Atorvastatin	Quebec, Canada (n = 19/38), >6 months	Reduced lesion MMP-13 expression and TGF-β signaling, no effect on AAA size	[135]
Atorvastatin[‡]	Hull, UK (n = 20/40), 4 weeks	No effect on MMP-2, -8 and -9 or TIMP-1 and -2 expression	[136]

[†]Number of patients receiving doxycycline or statin treatment versus total number of patients, or only treated patients, are quoted in parentheses.

[‡]Study with unexpected results.

[§]Patients treated with a few different statins or unspecified statins.

[¶]Patients used simvastatin, atorvastatin or pravastatin.

AAA: Abdominal aortic aneurysm; CRP: C-reactive protein; TIMP-1: Tissue inhibitor of metalloproteinase-1.