

## Matrix Metalloproteinases Promote Arterial Remodeling in Aging, Hypertension, and Atherosclerosis

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Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases, which are highly activated by inflammatory signaling, including that of angiotensin II (Ang II; Figure).<sup>1,2</sup> Activated MMPs are able to degrade collagen, elastin, and other extracellular molecules,<sup>2</sup> probably resulting in aging, hypertension, and atherosclerotic effects within the arterial wall (for reviews).<sup>1,2</sup> The modified extracellular matrix and its context of vasoconstrictors and vasodilators via cleavage by MMPs creates a proinflammatory microenvironment that shifts the phenotypes of endothelial cells (ECs) and vascular smooth muscle cells (VSMCs; for reviews).<sup>1,2</sup> These phenotypic shifts in which cells become secretory, migratory, proliferative and senescent facilitate arterial remodeling such as intimal-medial thickening (IMT), fibrosis, calcification, and aneurysms that are associated with a decrease in endothelial-dependent vasodilation and an increase in stiffness (for reviews).<sup>1,2</sup> Impressively, MMP inhibition effectively retards/alleviates arterial structural remodeling, decreases stiffness, and improves vascular endothelial function in animal models,<sup>3-8</sup> providing a rationale for the translation of MMP actions to therapeutic approaches in aging humans to curb the epidemic of cardiovascular disease.

### Role of MMP Activation in Arterial Remodeling

MMP activation facilitates arterial remodeling during aging (Figure; Table).

### Endothelial Inflammation

Increased MMP activity facilitates endothelial inflammation, such as EC senescence/apoptosis/necrosis, thrombosis, and dysfunction (Figure; Table). MMP-1 enhances EC senescence via p53 activation.<sup>9</sup> MMP-2 cleaves the intercellular and cell-matrix junctions, including vascular endothelial-cadherin and  $\beta$ - and  $\gamma$ -catenin, which initiates EC apoptosis or necrosis mediated by activated caspase 3, contributing to increased permeability.<sup>10,11</sup> Activated local MMP-2 also promotes platelet aggregation and thrombus formation, whereas loss of MMP-2 in platelets reduces arterial injury-associated thrombosis,<sup>38</sup> suggesting that MMP-2 is causally associated with formation

of thrombi. In addition, exposure of ECs to MMP-2 diminishes nitric oxide production, because of degradation of the heat shock protein 90, an endothelial nitric oxide synthase cofactor, and disturbs vasodilation.<sup>6</sup>

### Intimal-Medial Thickening

The MMP-associated invasion and proliferation of dedifferentiated VSMCs is an essential molecular and cellular event of diffuse IMT (Figure; Table). Intracellular activated MMP-2 markedly cleaves VSMC calponin-1, a differentiation marker, shifting the phenotype of these cells to a dedifferentiated state.<sup>12</sup> Extracellular-activated MMPs cleave collagen and basement membranes to release resident VSMCs from a non-permissive quiescent status to a permissive proliferation state (for review).<sup>39</sup> Activated MMP-1/-2/-9 treatment increases the release of soluble platelet-derived growth factor and milk fat globule-epidermal growth factor 8 (MFG-E8),<sup>40</sup> potent mitogens to VSMCs (for reviews).<sup>1,2</sup> MMP-2 increases phosphorylation of extracellular signal regulated kinase 1/2 (ERK1/2) and CDK-2/-4, promoting the proliferation of VSMCs.<sup>13,14</sup> In addition, the noncanonical MMP-1-protease-activated receptor-1-signaling cascade also triggers VSMC dedifferentiation and proliferation.<sup>41</sup> Notably, MMP-8 also directly promotes VSMC proliferation.<sup>42</sup> The exogenous broad spectrum MMP inhibitor, Batimastat, suppresses phosphorylation of ERK1/2,<sup>13</sup> and overexpression of the endogenous MMP inhibitor tissue inhibitor 3 of metalloproteinases reduces the proliferation of VSMCs.<sup>43</sup> These findings suggest that activated MMPs function as growth factors of VSMCs in vitro and MMP-mediated VSMC proliferation in vivo contribute to arterial IMT.<sup>31,41,42</sup>

The invasive property of VSMCs induced by MMPs is another essential cellular event of IMT. Ang II-signaling cascade molecules, Ang II per se, monocyte chemoattractant protein-1, MFG-E8, transforming growth factor (TGF)- $\beta$ 1, and intracellular calcium-dependent, calpain-1 all trigger the activation of MMP-2 in VSMCs (for reviews).<sup>1,2,44</sup> MMP-2 cleaves the basement membranes and enables VSMC invasion, which is prevented by the MMP inhibitor GM6001 (for reviews).<sup>1,2</sup> VSMCs isolated from both MMP-2 and MMP-9 knockout mice with reduced intimal hyperplasia lose their invasiveness in vitro.<sup>5</sup>

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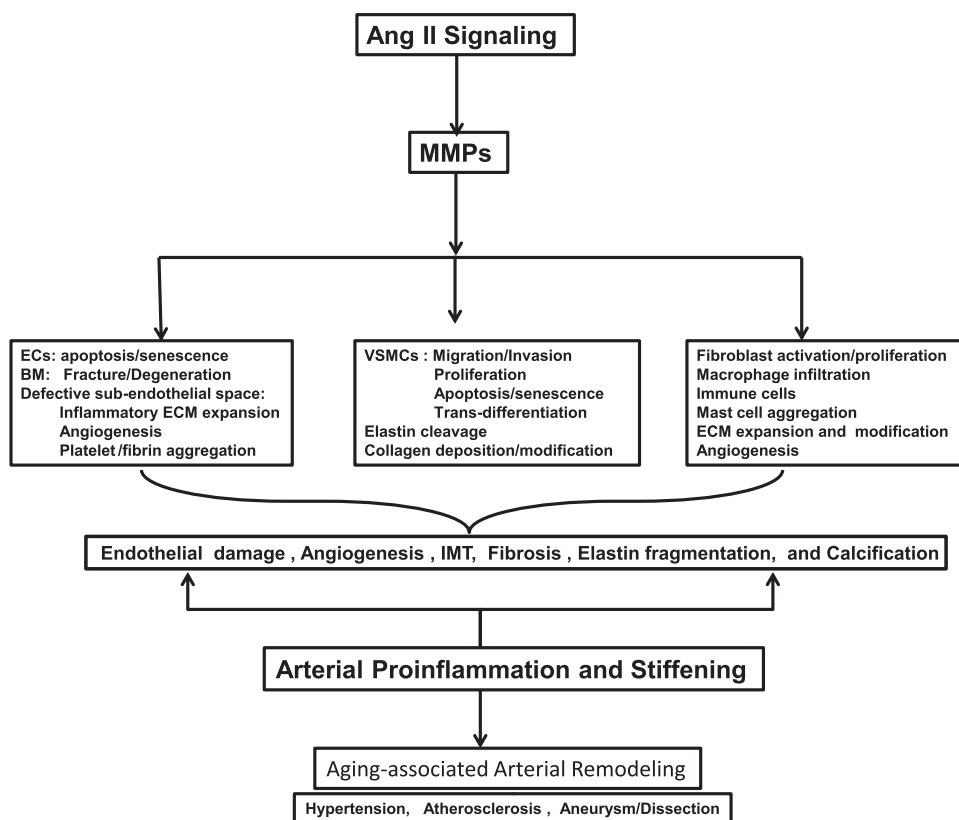
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## MMP Activity, Arterial Aging, and Arterial Disease



**Figure.** Matrix metalloproteinase (MMP) activation plays a central role in arterial proinflammatory remodeling and stiffening, which is a structural and functional signature of aging, hypertension, and atherosclerosis. BM indicates basement membrane; EC, endothelial cell; ECM, extracellular matrix; IMT, intimal-medial thickening; and VSMC, vascular smooth muscle cell.

### Elastin Fiber Network Destruction

MMP activation plays a causal role in the elastogenesis (production) and elastolysis (degradation) in the arterial wall (Figure; Table). Elastin fibers are effectively digested by activated MMP-2/-9.<sup>18</sup> An MMP activation inhibitor PD166793 markedly blunts elastin fiber degradation.<sup>3</sup> In addition, MMP activation triggers phosphorylation of ERK1/2, which retards tropoelastin production in VSMCs *in vitro* and elastogenesis in the rat aorta *in vivo*.<sup>13,45</sup> These findings suggest that proliferation in which phosphorylation of ERK1/2 is involved is coupled to the elastogenesis in VSMCs.

### Arterial Fibrosis

MMP-associated activation of TGF- $\beta$ 1 facilitates arterial fibrosis (Figure; Table). MMP cleavage of the elastin network releases fibrillin-1 bound latent TGF  $\beta$ -binding protein-1 (for reviews).<sup>1,2</sup> Also, MMP-2 cleaves the latent TGF  $\beta$ -binding protein-1 stepwise, leading to the activation of TGF- $\beta$ 1, enhancing increased VSMC production of collagen I, II, and III, and fibronectin (for reviews).<sup>1,2</sup> These data indicate that elastolysis and fibrosis may be 2 separate faces of the same coin. Activation of calpain-1 leads to MMP-2 activation, TGF- $\beta$ 1 activation and collagen production in VSMCs.<sup>27</sup> MMP-2/-9 is also closely associated with collagen accumulation in hypertensive arterial walls.<sup>19</sup>

MMP-8/-13, in contrast, is associated with collagen degradation in arterial wall plaques.<sup>28</sup>

### Calcification

MMP activation is associated with arterial calcification (Figure; Table). The overexpression of calpain-1 reduces the calcification inhibitors osteonectin and osteopontin and induces alkaline phosphatase activity via MMP-2 activation.<sup>27</sup> Collagenase pretreatment induces VSMC senescence.<sup>15</sup> Senescent VSMCs are phenotypically shifted into a secretory procalcification status in which calcification-related genes and proteins, including alkaline phosphatase activity, runt-related transcription factor 2, and bone morphogenic protein-1 become overexpressed (for review).<sup>46</sup> MMP-2/-9 deficiency retards the development of aortic calcification.<sup>8,18</sup>

### Adventitial Expansion

Activation of MMPs is associated with adventitial expansion and alterations to its properties (Figure; Table). MMP-2/-9 and their downstream target molecule, TGF- $\beta$ 1, activate adventitial (myo) fibroblasts, facilitating collagen production that contributes to adventitial fibroplasia.<sup>29,30</sup> An increased infiltration of monocytes/macrophages and mast cells into the adventitia accompanied by activation of MMP-2/-9 promotes the destruction of the arterial wall.<sup>32,33</sup>

**Table. MMP Activation is Involved in Arterial Remodeling and Functional Changes**

Arterial Remodeling and Functional Change	MMP
Endothelial inflammation-EC: apoptosis/senescence/death; and disruption of basement membrane	MMP-1/-2 <sup>9-11</sup>
Intimal-medial thickening-VSMCs: Proliferation/invasion/migration/apoptosis/senescence; and hypocontractility	MMP-1/-3 <sup>5,12-17</sup>
Elastolysis	MMP-2/-3/-9/-12 <sup>18-26</sup>
Fibrosis: collagen deposition	MMP-2/-8/-9/-13 <sup>27-30</sup>
Calcification	MMP-2 <sup>8,18,27</sup>
Adventitial expansion: fibroblast transdifferentiation into myofibroblasts; proliferation and inflammation; mast cell infiltration; and adipose tissue inflammation	MMP-2/-9/-12 <sup>30-33</sup>
Vasoconstriction: degraded $\beta$ 2-AR, eNOS, and CGRP as well as increased ET-1	MMP-1/-2/-9 <sup>4,6,34-36</sup>
Stiffening: tone	MMP-1/-7/-8/-9/-10/-12/-13/-15 <sup>29,30,34,37</sup>

AR indicates adrenergic receptor; CGRP, calcitonin gene-related peptide; EC, endothelial cell; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; MMP, matrix metalloproteinase; and VSMC, vascular smooth muscle cell.

### Arterial Wall MMP Activation During Aging, and in Hypertension or Atherosclerosis

The arterial remodeling mediated via MMP activation is the histopathologic signature of arterial aging, hypertension, and atherosclerosis (Figure).

#### Arterial Aging

Aging is the result of interactive genetic and epigenetic events within different cell types and tissues throughout a lifetime, and is characterized by a chronic low-grade arterial wall inflammation (proinflammation), stiffness, and a blood pressure (BP) increase (for reviews).<sup>1,2</sup> Proinflammation is not instigated by professional immunologic cells, such as leukocytes, but rather by phenotypically shifted arterial wall cells, including ECs, VSMCs, and fibroblasts (for reviews; Figure).<sup>1,2</sup> Proinflammation-associated arterial stiffening and BP increase are mainly determined by VSMC phenotypic shift or extracellular matrix modifications, or their combination.<sup>47</sup> Intrinsic stiffening of VSMCs increases with age, which is likely linked to activity of serum response factor.<sup>47,48</sup> Serum response factor gene inactivation reduces vasoconstriction and increases intrinsic elasticity in the carotid artery in the absence of a significant change in extracellular matrix.<sup>47</sup> Alteration of arterial stiffness in serum response factor–invalidated mice results from the downregulation of contractile protein genes, cytoskeletal organization, and in cell–matrix interactions.<sup>47</sup>

Aging enhances MMP-2/-7/-9/-14 activity in the aortic walls of rodents, nonhuman primates, and humans (for reviews).<sup>1,2</sup> Increased MMP activity is associated with increases in Ang II signaling, proinflammation, fibrosis, and elastin fragmentation (for reviews).<sup>1,2</sup> Ang II infusion to young

rats increases MMP-2 activation, IMT, and fibrosis in the arterial wall, similar to that of untreated old control rats (for review).<sup>1,2</sup> Importantly, an MMP inhibitor, PD166793, markedly blunts the age-associated increases in aortic MMP-2 and interstitial collagenase activity, and reduces the elastin fiber degeneration, collagen deposition, and BP increase.<sup>3</sup> Thus, Ang II/MMP-2 signaling is a driver of arterial wall matrix remodeling with aging, stiffening, and BP increase.

#### Hypertension

Aging increases the prevalence of hypertension (for reviews; Figure).<sup>1,2</sup> Hypertension is not only a disease characterized by increased BP but also characterized by proinflammation in the arterial wall. An increase in MMP proteolytic processes is one of the underlying mechanisms in hypertension.<sup>4,16,19,34,49-51</sup> In situ zymography demonstrates gradients in localization of activated MMP-2/-9, in particular in the diffusely thickened intima of hypertensive rats.<sup>4,16,19</sup>

Arterial MMP activation induction by Ang II plays a central role in the pathogenesis of hypertension. Exposure of carotid arterial rings to Ang II significantly increases MMP-2 activation, which is abolished by an AT1 antagonist Losartan.<sup>16</sup> MMP-2 activation is a constituent of Ang II–mediated BP elevation, which is dependent on the transcriptional control of MMP-7.<sup>50</sup> Knockdown of the MMP-7 gene attenuates both MMP-2 activation and BP elevation,<sup>50</sup> and as expected, MMP-2 inhibition by gene knockdown or pharmacological inhibition prevents a rise in BP in Ang II–induced hypertensive mice.<sup>50</sup>

Beyond Ang II stimulus, a mechanical and dietary sodium overload also increases the activation of arterial and circulating MMP-2/-9 gene and protein and its attendant hypertension.<sup>37,52</sup> Ex vivo data show that an increase in stress and disturbed blood flow in the arterial walls results in marked increases of MMP-2/-9 activity.<sup>53</sup> The BP-associated increases in arterial MMP-1/-2/-3/-9/-12 probably results from activation of the proinflammatory transcriptional factors nuclear factor- $\kappa$ B and v-ets avian erythroblastosis virus E26 oncogene homolog 1 (Ets-1).<sup>3,20</sup> In addition, in renal hypertension, the activation of MMP-2/-9 is dependent on increased BP, which contributes to increases in reactive oxygen species and decreases in NO bioavailability, as well as to eventual endothelial dysfunction.<sup>4</sup>

Importantly, the microprocessing of extracellular bioactive molecules via MMP activation reinforces the progression of hypertension (Table). Activated MMP-2 contributes to an increase in BP by both increasing the bioavailability of vasoconstrictors such as big endothelin-1 and decreasing vasodilators such as adventitial calcitonin gene-related peptide and endothelial nitric oxide synthase.<sup>4,6,35,36</sup> MMP-1/-2/-7/-9 reduces the density of  $\beta$  (2) adrenergic receptor in arterioles and enhances the arteriolar tone, which also contributes to an increase in BP.<sup>34,49</sup>

#### Atherosclerosis

Age-associated arterial wall remodeling provides fertile soil for the acceleration of atherogenesis (for reviews; Figure).<sup>1,2</sup> The MMPs, including MMP-2/-7/-8/-9/-13, are involved in the various stages of atherosclerosis, which are potentially associated with an increase in miR-21 and a decrease in miR-24, unique signatures of plaque instability.<sup>28,54-57</sup> miR-21 is an immunomiR, which is markedly increased

in atherosclerosis and regulates the associated immunoresponses.<sup>58,59</sup> Downregulation of miR-24 promotes an invasive macrophage subset.<sup>57</sup>

Strong evidence indicates that the MMP activation catalyzes atherosclerosis progression from a benign phenotype to fibroatheroma, characterized by a large and soft lipid-rich necrotic core and neovessel covered by a thin and inflamed fibrous cap (see review).<sup>60</sup> Increased uptake of glucose measured by positron emission tomography predicts MMP-9 activation, which is associated with high-risk lipid-rich, hemorrhage, or inflamed plaques.<sup>61</sup> Activated MMP-2/-7/-8/-9 promotes the angiogenesis, growth, and vulnerability of atherosclerotic plaques.<sup>54,62,63</sup> Importantly, the AT1 blocker Losartan inhibits MMP-2/-9 activation, neovascularization, and the vulnerability of atherosclerotic plaques.<sup>62</sup>

### Arterial Aneurysm/Dissection

Age-associated hypertensive or atherosclerotic remodeling sets the stage for inflammatory catastrophes: aortic dissection and rupture (Figure). In humans, activation of MMP-9 is higher in dissected arterial than in control tissue<sup>64</sup>; and is closely associated with the progression of aneurysm.<sup>64,65</sup> In mice, optical imaging of MMP activity accurately predicts the growth rate of abdominal aortic aneurysm (AAA).<sup>66</sup> These findings suggest that inflammatory MMP signaling pathways play a central role in the pathogenesis of aortic aneurysm/dissection.

The Ang II receptor AT1 antagonist Losartan, and TGF receptor mutant potentially prevent the formation and progression of aneurysm in various mouse models and alleviate activation of MMP (protein and gene).<sup>21,30,67-73</sup> MMPs, downstream molecules of both Ang II and TGF- $\beta$  (for reviews),<sup>1,2</sup> play causal roles in the development of the aortic aneurysm.<sup>22,23,66</sup> MMP-2/-9 activation is able to erode the arterial wall, a histological foundation of the aortic aneurysm development.<sup>22,66</sup> Furthermore, MMP-2/-9 activation signaling also promotes a loss of VSMCs and facilitates aortic dilation.<sup>17</sup> Impressively, MMP-2 regulates a TGF- $\beta$ 1/ERK1/2 noncanonical pathway in VSMCs and facilitates vascular wall dilatation in a fibrillin-1 mutation Marfan syndrome animal model.<sup>23</sup>

Emerging evidence indicates that the miRs are strong modulators of the Ang II, TGF- $\beta$ 1, MMP-mediated cascade and promote aneurysm formation. Notably, an age-associated increase in miR-29b upregulates the activity of MMP-2/-9 gene and protein, activates TGF- $\beta$ 1, and promotes aortic dilation in both elastase infusion and Ang II infusion aneurysmal models.<sup>67,70</sup> In addition, miR-21 is implicated as a modulator of proliferation and apoptosis of VSMCs and MMP-2 gene expression during development of AAA in apolipoprotein E<sup>-/-</sup> mice induced by Ang II.<sup>73</sup> A reduction of miR-195 in AAA is associated with the upregulation of MMP-2/-9 gene and protein, promoting the AAA development in an apolipoprotein E<sup>-/-</sup>-infused Ang II model.<sup>72</sup>

Analysis of proteins extracted from aortic media detected an amyloid protein called medin, a 5.5 kDa fragment of MFG protein E8, a downstream molecule of Ang II, which becomes deposited in the aortic medium in  $\approx$ 100% of the white population by 50 years of age (for reviews).<sup>1,2,44</sup>

Importantly, the medin precursor of MFG-E8 is also involved with the progression of both atherosclerosis and hypertension such as arterial stiffening (for reviews).<sup>2,44</sup> The medin fragment derives from the C2-like domain of MFG-E8 and is colocalized with elastin fibers of arteries and is associated with the development of aortic aneurysm/dissection (for reviews).<sup>1,2,44</sup> In an in vitro model, aggregated medin induces death of VSMCs and increased the production of metalloproteinase-2, probably promoting the destruction of the arterial wall in vivo (for reviews).<sup>1,2,44</sup> In addition, an increase in BP through upregulation of NF- $\kappa$ B and Ets-1 and activation of MMP-2/-3/-9/-12 accelerates AAA in rats induced by an elastase perfusion.<sup>3,20</sup>

Cellular-specific inflammatory changes also promote the formation of aortic aneurysm/dissection. EC-specific Nox-2 associated reactive oxygen species production increases MMP activation and susceptibility to aortic dissection.<sup>24</sup> Adventitial mast cells directly augment MMP-9 activity produced by monocytes/macrophages, contributing to the progression of AAA in the apolipoprotein E<sup>-/-</sup> mice.<sup>32</sup> Pharmacological inhibition with Tranilast, an inhibitor of mast cell degranulation, attenuates AAA development in these rodents.<sup>32</sup>

Importantly, ERK1/2 deficiency reduces MMP-2/-9 activation and prevents AAA induced by elastase infusion.<sup>25</sup> Cathepsin S deficiency decreases MMP-2 activation and retards the pathogenesis of aneurysm.<sup>26</sup> Chronic treatment with a broad-spectrum MMP inhibitor, doxycycline, prevents the development of spontaneous aortic aneurysm lesions.<sup>22</sup>

### Concluding Remarks and Perspective Views

A chronic increase in MMP activation is central to age-associated arterial structural remodeling (Figure). Furthermore, the quintessential vascular diseases, such as hypertension and atherosclerosis could be viewed as accelerated arterial aging and are also linked to increased MMP activation (Figure). The pathophysiological vascular, cellular and molecular events of aging, hypertension, atherosclerosis, and their complications are recapitulated in experimental animals with overactivation of MMPs (Table). In contrast, MMP inhibition attenuates age-associated pathophysiologic arterial remodeling in animal models of hypertension and atherosclerosis.<sup>4-8,22</sup> Thus, appreciation of the MMP involvement in arterial wall aging and age-associated arterial diseases and the ability to detect its involvement in arterial wall inflammation, vulnerable plaques, and the progression of aneurysm/dissection will affect future translational research to delay the arterial remodeling that accompanies aging and its participation in these age-associated arterial diseases.

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### Disclosures

None.

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