Matrix Metalloproteinase-9: Many Shades of Function in Cardiovascular Disease

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Matrix metalloproteinase (MMP)-9, one of the most widely investigated MMPs, regulates pathological remodeling processes that involve inflammation and fibrosis in cardiovascular disease. MMP-9 directly degrades extracellular matrix (ECM) proteins and activates cytokines and chemokines to regulate tissue remodeling. MMP-9 deletion or inhibition has proven overall beneficial in multiple animal models of cardiovascular disease. As such, MMP-9 expression and activity is a common end point measured. MMP-9 cell-specific overexpression, however, has also proven beneficial and highlights the fact that little information is available on the underlying mechanisms of MMP-9 function. In this review, we summarize our current understanding of MMP-9 physiology, including structure, regulation, activation, and downstream effects of increased MMP-9. We discuss MMP-9 roles during inflammation and fibrosis in cardiovascular disease. By concentrating on the substrates of MMP-9 and their roles in cardiovascular disease, we explore the overall function and discuss future directions on the translational potential of MMP-9 based therapies.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases responsible for both physiological and pathophysiological tissue remodeling. MMPs cleave all structural elements of the extracellular matrix (ECM), as well as process a variety of non-ECM substrates. Currently, there are 25 family members described in vertebrates, with 22 found in humans. MMPs were initially provided with descriptive names, based on substrate specificity, and were classified into five groups: collagenases, gelatinases, stromelysins, matrilysins, and membrane type (114). A numbering system corresponding to the order of discovery was adapted after it was realized that more MMPs existed than originally expected.

Normal myocardium possesses a number of ECM proteins, including collagens, laminins, fibronectin, and low levels of matricellular proteins, all of which play a role in the physiological performance of the heart. Collagen, the most abundant ECM protein, forms a complex network to provide three-dimensional structure and tensile strength to the cardiac muscle fibers. In cardiovascular disease, the cardiac muscle is subjected to tissue remodeling to preserve cardiac function and integrity, which involves breakdown of the collagen network. The MMPs that can cleave collagen include MMP-1, -2, -8, -9, and -14 (31, 109).

MMP-9, first termed 92-kDa type IV collagenase or gelatinase B, plays a major role in the degradation of ECM in a large spectrum of physiology and pathophysiology processes that involve tissue remodeling. For example, MMP-9 expression is important for embryo implantation, starting from the trophoblastic invasion during the early gestation period (12). MMP-9 is present in developing cardiac tissue in humans and rodents, and is expressed between 16 and 18 days of embryogenesis (61, 99). MMP-9 is also reported to play a significant role in neovascularization through the proteolytic degradation of the proteins in basal lamina of the blood vessels and a release of the biologically active form of vascular endothelial growth factor (6).

MMP-9 plays important roles in immune cell function. MMP-9 deletion promotes the recruitment of eosinophils and Th2 cells into the lungs during allergen challenge (74). In pathophysiological conditions, MMP-9 is upregulated during development and wound healing, as well as during pathologies that involve inflammatory processes, including arthritis, diabetes, and cancer (38). In these pathophysiological conditions, MMP-9 proteolytic properties contribute to stimulate the immune response to initiate pathogenesis and exacerbate disease progression. MMP-9 robustly increases during several cardiovascular diseases, including hypertension, atherosclerosis,

and myocardial infarction (MI). The large number of publications on MMP-9 highlight the importance of this enzyme in the list of prospective and important biomarkers, which could be used in combination with other biomarkers to improve diagnosis or accelerate drug discovery (38). In this review, we discuss the structure, activity, and regulation of MMP-9, as well as its roles in common cardiovascular pathologies and potential for translational applications.

MMP-9 Structure and Activity

MMP family members share similar fundamental structural characteristics and are classified according to their substrate specificity. By this classification, MMP-9 belongs to the gelatinase subgroup and is known as gelatinase B due to its ability to degrade gelatin.

Human MMP-9 consists of an NH_2-terminal pro-domain, a catalytic domain, a linker domain, and a COOH-terminal hemopexin-like domain that combine to form a 92-kDa pro- and 88-kDa active enzyme in humans (90). The catalytic domain of MMP-9 contains two zinc ions, five calcium ions, and three repeats homologous to the type II module of fibronectin. One of the two zinc ions of the catalytic domain and cysteine switch motif of the pro-domain are structurally coordinated to keep MMP-9 inactive (108). The catalytic zinc ion is essential for proteolytic activity.

MMP-9 has a unique domain termed the fibronectin-like domain, which consists of three repeats of fibronectin type II of ~58 amino acids. This domain is heavily O-glycosylated and contains elongated linker between catalytic and hemopexin-like domain (89). The fibronectin-like domain is essential in binding to denatured collagen or gelatin (83). Hemopexin-like domain shares sequence similarity to plasma hemopexin and is present in MMP-9. In pro-MMP-9, the hemopexin-like domain forms a tight complex with TIMP-1 and TIMP-3 though their COOH-terminal domains (79). Pro-MMP-9 is complexed with TIMP-1 in the Golgi apparatus of the cell before secretion (104). TIMP-1 is bound to the pro-MMP-9 via COOH-terminal domain, leaving the NH_2 terminus capable of inhibiting other MMPs.

MMP-9-null and MMP-9 overexpression mice have been developed. Mouse MMP-9 shares 72% identity and 99% homology with human MMP-9. Human MMP-9 contains a cysteine residue at the 87 amino acid that permits it to bind to neutrophil gelatinase-associated lipocalin, whereas mouse MMP-9 has a serine in this domain (54, 70, 136). Mouse MMP-9 contains 23 extra amino acids,
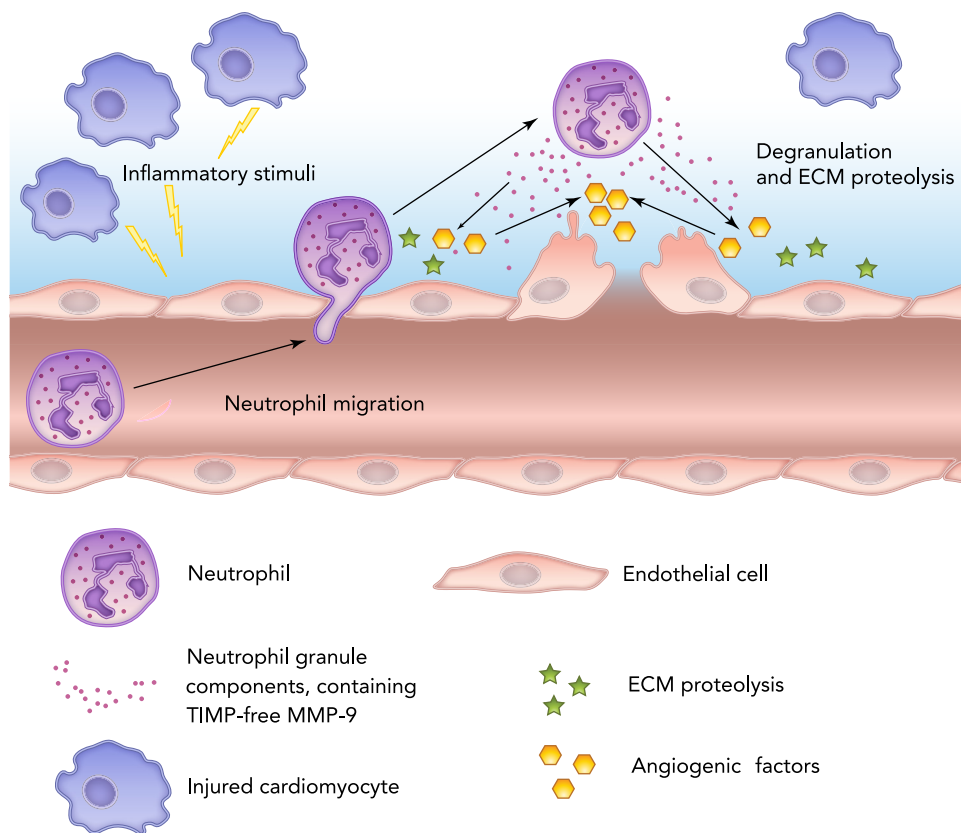

FIGURE 1. Schematic representation of neutrophil roles in inflammation and MMP-9 release

Inflammatory stimuli

Degranulation and ECM proteolysis

Neutrophil migration

Neutrophil

Neutrophil granule components, containing TIMP-free MMP-9

Injured cardiomyocyte

Endothelial cell

ECM proteolysis

Angiogenic factors

mainly between *amino acids 486–501* and *705–711*. This results in mouse MMP-9 having an apparent molecular weight of 105 kDa (pro-) and 95 kDa (active).

Cell Expression of MMP-9

MMP-9 is secreted by a wide number of cell types, including neutrophils, macrophages, and fibroblasts. Neutrophils contain multiple proteases, such as serine proteases (elastase, cathepsin G, and proteinase 3), MMPs (MMP-8 and -9), and urokinase plasminogen activator (uPA). All proteases released from neutrophils promote MMP-9 activation (118). In neutrophils, MMP-9 is synthetized during granulocyte differentiation in the bone marrow. In humans but not rodents, neutrophil MMP-9 is covalently linked with lipocalin, which protects it from proteolytic degradation (21, 52). MMP-9 degrades ECM with subsequent activation of major proangiogenic factors such as vascular endothelial growth factor and fibroblast growth factor-2 (FIGURE 1) (4).

Macrophages are a potent source of MMP-9. Fang and colleagues showed that differentiated macrophages from circulating monocytes isolated from patients with acute MI or stable angina had a twofold increase in mRNA and protein levels of MMP-9 compared with the control groups (25). Monocyte entry into the tissue delineates the transition into the macrophage, at which time MMP-9 expression increases. The release of MMP-9 by macrophages in apoE-deficient mice greatly enhanced elastin degradation and induced plaque disruption (FIGURE 2). Among the macrophage phenotypes, foam cells are a predominant source of active MMP-9 (81). In MI, MMP-9-deficient mice showed reduced rupture rate and attenuated ventricular dilation, which was associated with reduced macrophage infiltration (23).

Siwik and colleagues showed an abundance of MMP-9 in cardiac fibroblasts during oxidative stress (112). Cardiac fibroblasts express MMP-9 after stimulation with IL-1β and TNF-α through ERK1/2 and nuclear factor-κB (NF-κB) signaling pathways (11). The activation of MMP-9 expression in cardiac fibroblasts is concomitant with a decrease in collagen synthesis rates to stimulate a net collagenolytic environment. MMP-9 is also actively involved in the cardiac fibroblast migration. Wang et al. showed that cardiac fibroblasts treated with a recombinant protein encoding only the catalytic domain of MMP-9 stimulated cardiac fibroblast migration, increased collagen synthesis, upregulated
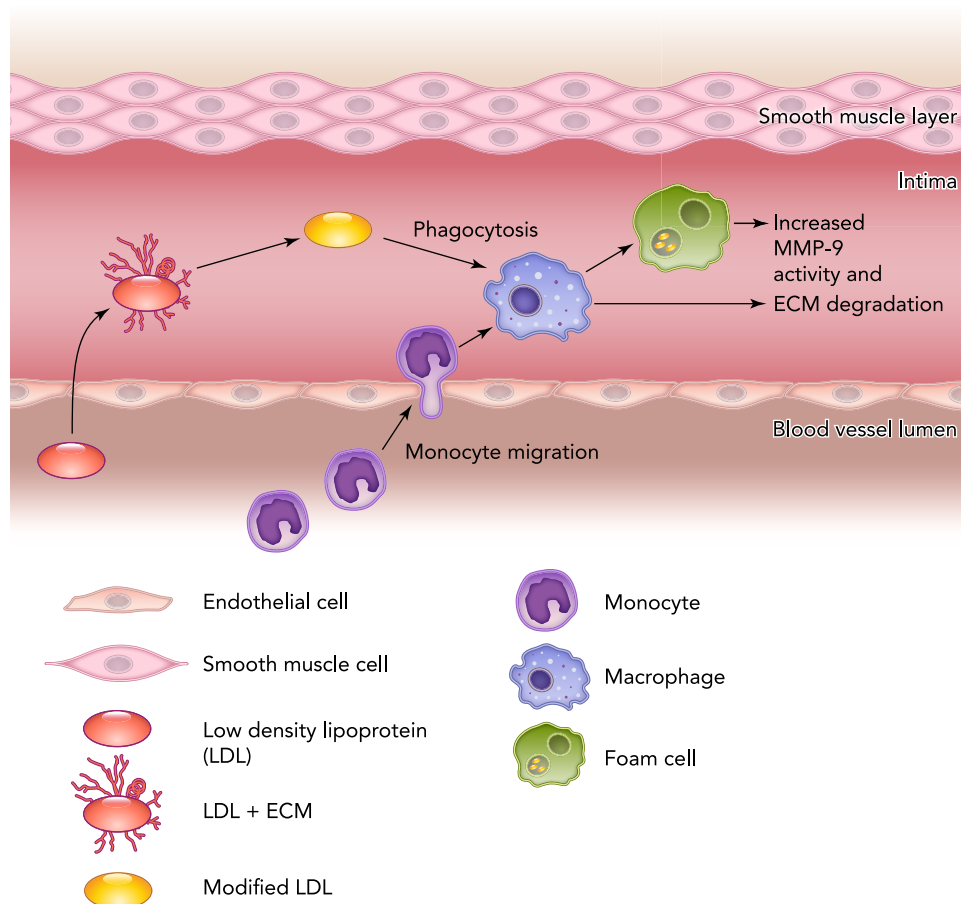

FIGURE 2. **Schematic representation of macrophage roles in inflammation and MMP-9 release**

angiogenic factors, and induced the transition of cardiac fibroblasts to myofibroblasts (128). The role of the myofibroblasts is essential in post-MI healing. Myofibroblasts produce ECM and are able to contract, thus contributing to tissue replacement and scar formation post-MI (FIGURE 3) (121).

Regulation of MMP-9

At the transcriptional level, MMP-9 is positively regulated by multiple factors, including E-26 (Ets) transcription factors, NF-κB, polyomavirus enhancer A-binding protein-3 (PEA3), activator protein-1 (AP-1), specificity protein 1 (Sp-1), and serum amyloid A-activating factor (SAF)-1 (16) (FIGURE 4).

Ets are a family of transcription factors associated with a variety of biological functions including cellular differentiation, cell migration, proliferation, apoptosis, and angiogenesis. Ets are capable of inducing MMP-9, along with uPA and integrins β2 and β3 (85).

NF-κB is capable of binding to κB DNA on the promoters or enhancers of genes to regulate expression (13). Bond et al. reported an increase in MMP-9 levels in vascular smooth muscle cells via NF-κB mechanisms (8). Inhibition of transcription factor NF-κB reduces MMP-9 production in vascular smooth muscle cells and macrophages (8, 37). Reactive oxygen species can activate MMP-9, both directly and indirectly, by activating transcription factors such as NF-κB (87). Ang II has direct and indirect effects on the expression of MMP-9. In ventricular myocytes, Ang II directly stimulates NF-κB to induce MMP-9 expression (107). Ang II activates epidermal growth factor receptor and the mitogen-activated protein kinase pathway to induce MMP-9 expression (111). Aldosterone, which is produced locally in the myocardium, triggers MMP-9 production through a NF-κB mechanism (67), as does the matricellular protein osteopontin (97).

PEA3 and AP-1 binding sites are present in the MMP-9 promoter (129, 132). AP-1 has two binding sites on MMP-9, and activation of MMP-9 is preceded by a rapid transient increase in AP-1 protein levels (131). Thrombospondins stimulate production of MMP-9 by activating AP-1 (36). Donnini and colleagues showed that fragments of thrombospondin
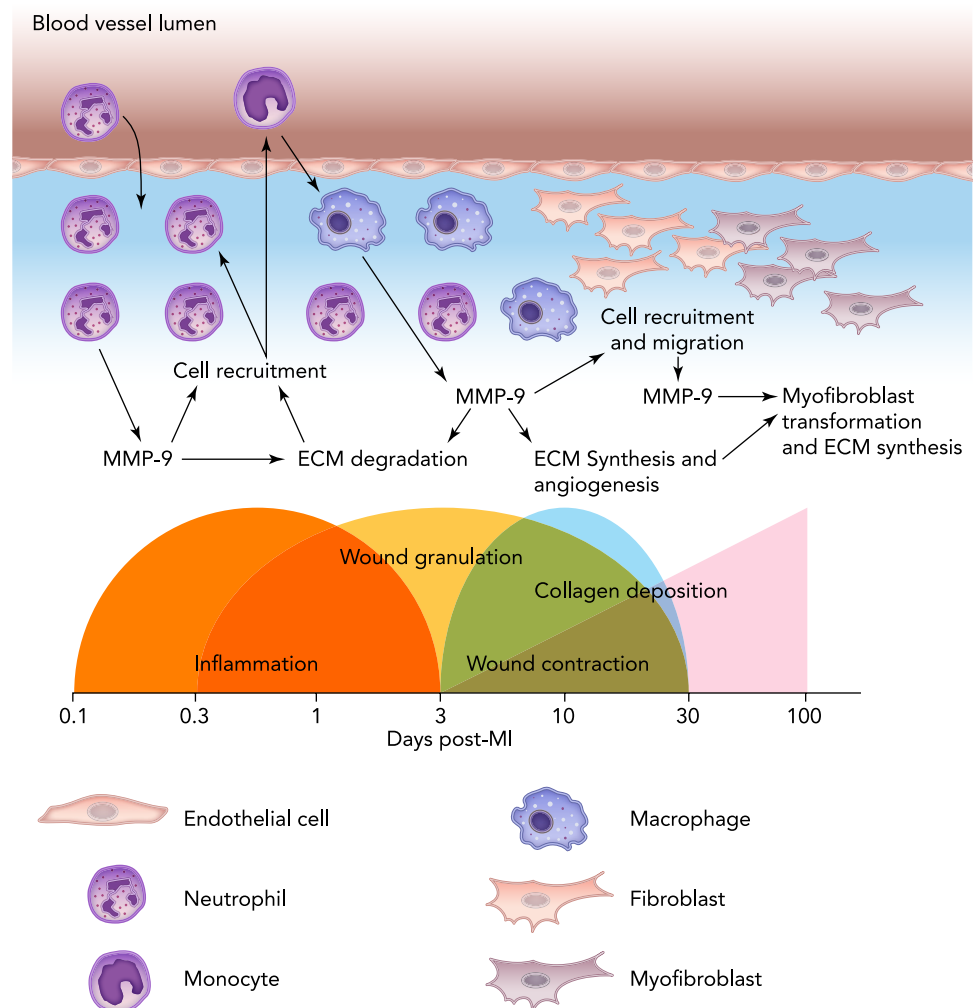

FIGURE 3. Schematic representation of the cells involved in the post-MI inflammatory and wound-healing process

promote MMP-9 production in bovine capillary endo-thelial cells (22).

Sp-1 binds to the MMP-9 promoter to induce transcription. Sp-1 undergoes several posttran-scriptional modifications such as phosphorylation and glycosylation to increase transcription (78, 115). Inhibition of Sp-1 leads to decreased MMP-9 expression (78).

In addition, the transcription factors described above cross-interact to regulate MMP-9 expression, which may be especially important in vivo. In vascu-lar smooth muscle cells, upregulation of MMP-9 is mainly attributed to expression and activation of NF-κB and AP-1 transcription factors (120). Occupa-tion by AP-1 alone, however, is not sufficient for maximal MMP-9 transcription, and the cooperation of either NF-κB or Sp-1 binding proteins upstream of the AP-1 site is required for full MMP-9 transcription induction (5). SAF-1 is an inflammatory responsive transcription factor that induces MMP-9 transcrip-tion via cooperation with AP-1 (101). In the MMP-9 promoter region, SAF-1 is located in close proximity to AP-1 elements. Mutation of either SAF-1 or AP-1 greatly affects induction of the MMP-9 promoter and reduces the ability of SAF-1 and AP-1 to activate transcription (101).

Among the cytokines capable of regulating MMP-9 expression, an important role is assigned to TNF-α. Alexander and Acott showed that TNF-α triggers the production of MMP-9 through the pro-tein kinase C signal-transduction pathway (2). Lau et al. showed TNF-α upregulated MMP-9 expres-sion in coronary arteries (62, 63). Heat shock pro-tein 60 has been shown to stimulate TNF-α followed by MMP-9 production in macro-phages (58). In rat embryonic cardiomyoblast cell line H9c2, the NF-κB II binding site within the promoter region of MMP-9 ($-626/-617$) plays a key role in upregulation of MMP-9 expression by TNF-α induction (133). Among other cytokines ca-pable of inducing MMP-9, IL-1β was shown to increase NF-κB and AP-1 in rat myocytes (69). MMP-9 expression has been reported in cardiomy-ocytes (41).

Classical MMP-9 activation includes disruption of the interaction between the zinc molecule in the catalytic domain and the cysteine switch in the pro-domain. This structural modification leads to cleavage of the pro-form and production of active enzyme. MMP-9 is activated by other MMPs, in-cluding MMP-2, -3, -13, -17, and -26 (32, 55, 86, 119). For example, activation of pro-MMP-3 by plasmin, which is generated from plasminogen by uPA bound to the uPA receptor on the plasma membrane, leads to activation of pro-MMP-9 (96). Proteolytic enzymes, such as plasmin, urokinase-type plasminogen activator, and tissue-type plas-minogen activator, are capable of cleaving the pro-domain to activate MMP-9 (92).

Another example of indirect pro-MMP-9 activa-tion is through the initial activation of MMP-2 and -13 on the cell surface by membrane type-1-MMP (32). Several serine proteases are capable of pro-MMP-9 activation. Tissue-associated chymotryp-sin-like proteinase activates pro-MMP-9 in skin tissues from chronic unhealed wounds (40). Pan-creatic trypsin-2 from human carcinoma was an effective pro-MMP-9 activator (113). Posttransla-tional modifications of MMP-9 are another potent mechanism of increasing extracellular MMP-9 ac-tivity, and these include S-nitrosylation and N-gly-cosylation. MMP-9 has one S-nitrosylation site at cysteine and two N-glycosylation sites at aspar-agines in positions 38 and 120 (59, 72).
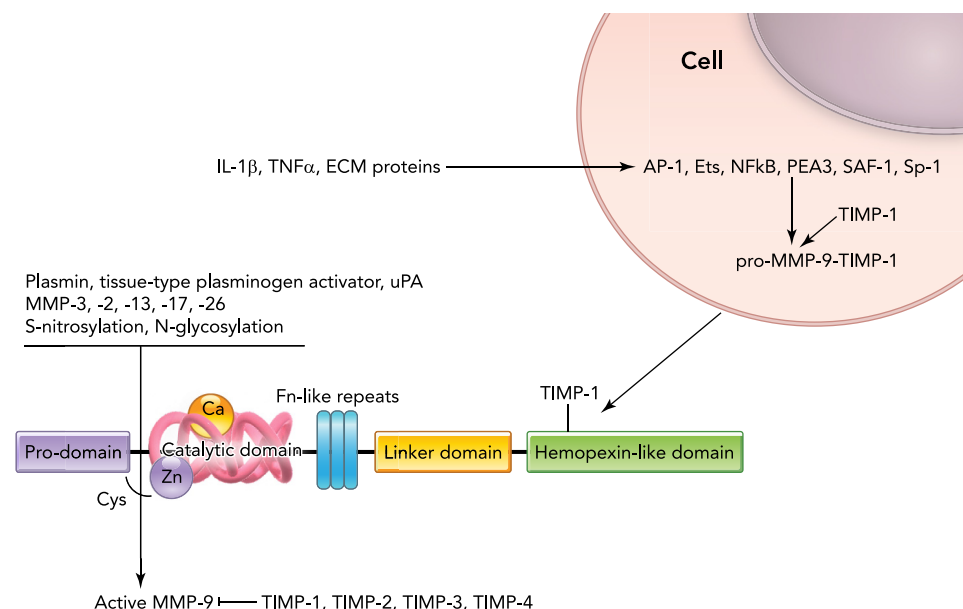

FIGURE 4. MMP-9 structure and factors regulating MMP-9 transcription and translation
Ets, E-26 transcription factor; NF-κB, nuclear fac-tor κB; PEA-3, polyomavirus enhancer A-binding protein-3; AP-1, activator protein 1; SAF-1, serum amyloid A-activating factor 1; HSP60, heat shock protein 60; TIMP, tissue inhibitor of metalloprotei-nase; uPA, urokinase plasminogen activator.

Table 1. *Gene polymorphisms associated with increased MMP-9 expression and cardiovascular disease*

Disease	Gene Polymorphisms
Hypertension	C-1562T, R279Q, 836GA
Atherosclerosis	C-1562T, a number of microsatellite (CA)$_n$ repeats of >22, R279Q
Myocardial infarction	C-1562T, R279Q, R668Q

Gene polymorphisms associated with increased MMP-9 expression and cardiovascular disease (15, 21, 38, 43, 56, 76, 80, 96–97, 107, 112, 114).

Although MMP-9 is synthesized and secreted in a pro-form, there is evidence that MMP-9 may also be activated intracellularly. Pereira and colleagues reported that activated MMP-9 accumulates in cells undergoing apoptosis, although this does not rule out the possibility that MMP-9 had been activated extracellularly and taken back up (93). Tissue inhibitor of metalloproteinase (TIMP)-1-free MMP-9 has been shown to accumulate in microvascular endothelial cells in endothelial vesicles after phorbol myristate acetate stimulation (82). Future studies are warranted to determine whether MMP-9 can be activated intracellularly.

Inhibition of MMP-9 is performed by TIMPs binding to the zymogen forms of the enzyme (34). All TIMPs are known to interact with MMP-9 and inhibit its activity (9). TIMP-1 binds to pro-MMP-9, in addition to inhibiting its active form (104). In circulation, α_2 macroglobulin inhibits MMP-9 to prevent systemic MMP-9 activation.

MMP-9 Roles in Cardiovascular Disease

Cardiovascular diseases involve inflammation and altered tissue remodeling associated with the reorganization of ECM and the activation of MMP-9.

MMP-9 Gene Polymorphism in Cardiovascular Disease

T allele carriers of C-1562T gene polymorphism are associated with an increased level of blood pressure and aortic stiffness in a hypertensive population (146) (Table 1). Carriers for the R279Q polymorphism show susceptibility to the increase in aortic stiffness and an increased risk for the development of hypertension (138). Another MMP-9 gene polymorphism, 836GA, alone and in haplotype with C-1562T, was shown to contribute to the development of hypertension and aortic stiffness (71).

Genetic variation in promoter polymorphisms imposes allele-specific effects on the gene expression of MMP-9 (50, 144). In particular, the C-1562T polymorphism has been reported to increase gene expression of MMP-9 and was associated with severity of coronary atherosclerosis. The individuals carrying the T allele were predisposed to increased plaque instability through increased ECM degradation. Another

functional polymorphism in the promoter region of MMP-9 is microsatellite (CA)$_n$. (CA)$_n$ is located near the −90 position, which corresponds to a sequence of cytosine-adenine repeats (20). The number of CA repeats of 22 or more in the microsatellite of MMP-9 promoter was associated with carotid atherosclerosis and the formation of plaques with a thin fibrous cap (28). A combination of C-1562T in the promoter region and a G/A transition in exon 6 (R279Q) was reported to be a major haplotype among the Caucasian population with different stages of atherosclerosis (100).

The C-1562T polymorphism in the MMP9 gene promoter is associated with an elevated MMP-9 expression and increased susceptibility to MI (56, 127). The R279Q polymorphism of MMP-9 gene is not independently associated with MI; however, in combination with smoking, it has a synergistic effect and is significantly associated with the risk
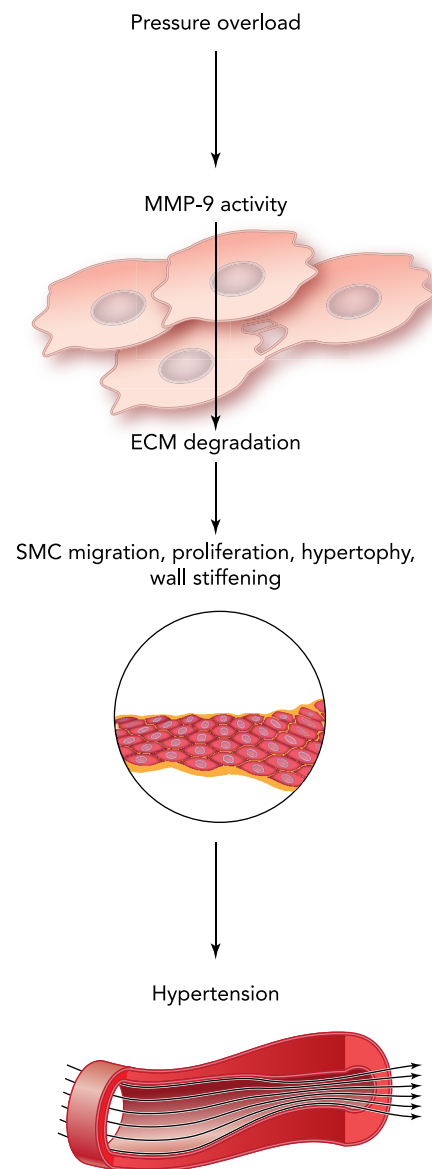

Pressure overload

↓

MMP-9 activity

↓

ECM degradation

↓

SMC migration, proliferation, hypertophy, wall stiffening

↓

Hypertension

FIGURE 5. **Schematic representation of MMP-9 involvement in hypertension**

for MI (126). Another polymorphism in MMP-9, R668Q has been suggested to relate to the development of MI; however, Rodius and colleagues evaluated 1,049 patients and showed the frequency of MMP-9 R668Q was ~25% and was not increased in patients with MI (105). The C-1562T R668Q polymorphisms in the MMP-9 gene also have been associated with heart failure (HF) and mortality (70).

Hypertension

Hypertension is defined clinically as increased arterial blood pressure over 139 systolic and/or 89 diastolic mmHg. Hypertension is associated with alterations in cardiac, renal, neural, and vascular control systems which increase cardiac output, arterial stiffness, and peripheral resistance. Hypertensive patients express higher levels of MMP-9 in serum, which positively correlate with aortic stiffness (FIGURE 5) (116). MMP-9 activity is induced very early with the development of hypertension, contributing to collagen breakdown and arterial distensibility. An increase in fibrillar collagen in the compensated stage of hypertension is associated with increased MMP-9 activity (130). An increased arterial pressure and altered remodeling in the blood vessels lead to a pressure overload of the heart. Under these conditions, both vascular and cardiac tissues undergo additional compensatory remodeling. MMP-9 activity is increased in arteries with high pressure compared with vessels under normal pressure (64). Compensatory cardiac hypertrophy that develops in response to an increased pressure overload in hypertension is an established risk factor for atrial fibrillation, diastolic and systolic HF, and sudden death (53). Compensatory hypertrophy of the heart is associated with increased MMP-9 activity. Li et al. showed increased MMP-9 activity during compensatory hypertrophy in spontaneously hypertensive rats (66). ECM degradation increases during the transition stage from compensation to clinically apparent HF and associates with increased MMP-9 activity (1).

Atherosclerosis

Atherosclerosis is defined clinically as buildup of fats and cholesterol in the arterial wall, which progresses to a plaque formation and restriction of blood flow. Human atherosclerotic plaques contain mostly collagen type I, III, IV, V, XI, and XVI (102). MMP-9 plays divergent roles in the formation and destabilization of atherosclerotic plaques (FIGURE 6A) (80). Plaque ruptures are associated with increased MMP-9 proteolytic activity (10, 60). Arterial remodeling is also associated with increased collagen I and IV accumulation and degradation. Flamant et al. reported increased deposition of collagen IV in the carotid arteries of

MMP-9-null mice treated with Ang II, along with reduced arterial compliance, compared with wild-type mice (29). In the plaque, the major source for MMP-9 is macrophage-derived foam cells, and MMP-9 associates with the formation of a vulnerable thin fibrous cap (125). In apolipoprotein E-null mice, MMP-9 levels were highly correlated with incidence of plaque rupture (35). An increase in systemic MMP-9 levels is highly correlated with cardiovascular mortality in patients with atherosclerosis (7). Serum MMP-9 levels correlate with C-reactive protein, interleukin-6, and fibrinogen levels and serve as an identification marker for patients at risk for future MI (26, 47). These findings suggest deleterious effects of the MMP-9 over-expression on the progression of atherosclerosis. Interestingly, MMP-9-null mice show increased deposition of fibrin, which suggests that fibrin is degraded by MMP-9 (65). In atherosclerosis, increased levels of plasminogen activator inhibitor
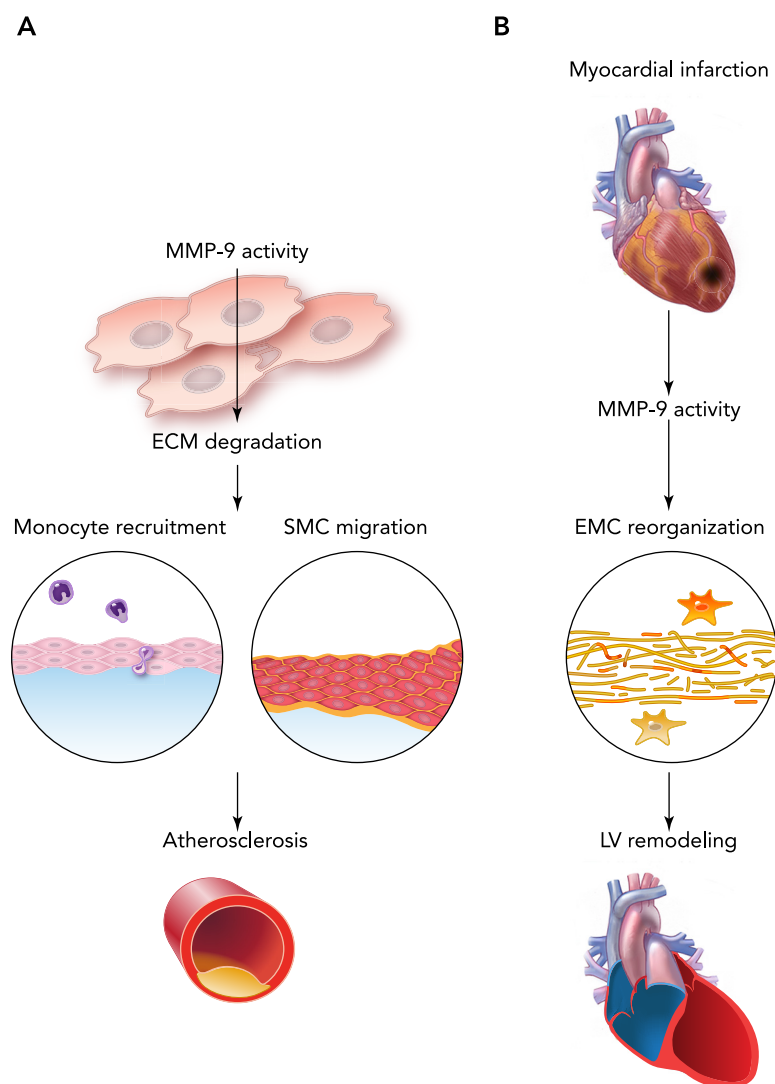

FIGURE 6. **Schematic representations of MMP-9 involvement**
Schematic representation of MMP-9 involvement in the atherosclerosis (A) and MI (B).

Table 2. *A partial list of ECM, chemokines, cytokines, and substrates degraded, activated or inactivated by MMP-9*

MMP-9	Substrate
Degrades	Collagen type I, II, III, IV, V, XI, XVI, fibronectin, laminin, osteopontin, thrombospondin-1, tenascin-C, galectin-3, decorin
Activates	CXCL5, CXCL8, TNF-α, IL-1β, TGF-β
Inactivates	CXCL1, CXCL4, CXCL5, CXCL7, CXCL12, IL-1β

A partial list of ECM, chemokines, cytokines, and substrates degraded, activated or inactivated by MMP-9 (20, 23, 25, 31, 33–34, 36, 54, 58–59, 61, 64, 79, 84, 89, 92-92, 106, 109, 111). IL, interleukin; TNF-α, tumor necrosis factor α; TGF-β, transforming growth factor β; CXCL, chemokine (C-X-C motif) ligand.

accelerate the process by allowing fibrin deposition in developing lesions. Since MMP-9 levels are reduced in plasminogen-deficient mice, it is possible that plasminogen regulates fibrin through MMP-9 signaling (17). Thus MMP-9 signaling may contribute to a reduction in thrombus size and play a beneficial role in suppressing the progression of atherosclerosis by inhibiting fibrin deposition.

MI

MI is an acute event characterized by irreversible myocardial tissue injury that develops after prolonged disruption of blood supply to the myocardium. MI is characterized by increased inflammation that replaces necrotic tissue with a fibrotic scar (134). Type III collagen increase is observed as early as 2 days post-MI in rats and is aimed to preserve the integrity of the injured myocardium (15). The ratio of collagen deposition post-MI shifts toward the predominance of collagen I (FIGURE 6B) (147). All of these adverse changes in collagen deposition are observed in HF in vivo models and in humans. Higher MMP-9 levels play an important role during the early stages of acute MI and progression to HF, when observed in decompensated patients (51). MMP-9 levels increase as early as several minutes post-MI and remain increased for the first week in many animal models of MI (24, 106). The early increases in MMP-9 levels post-MI correlated with increased numbers of neutrophils, and later increases at *days 2–4* with the infiltration of macrophages. These changes show an important role of MMP-9 in different stages of the inflammatory response. MMP-9-null deletion reduces the number of macrophages post-MI leading to attenuated enlargement of the left ventricle (LV) and reduced collagen accumulation (23). Targeted MMP-9 deletion in mice stimulates neovascularization and improves LV remodeling in the permanent occlusion model of MI (68). Interestingly, transgenic overexpression of MMP-9 in the macrophages unexpectedly shows improved cardiac function and attenuated inflammatory response at *day 5* post-MI in mice, suggesting that MMP-9 regulates both macrophage pro- and anti-inflammatory phenotypes and contributes to LV remodeling (142).

MMP-9 Substrates

Cardiac and vascular remodeling include the reorganization of ECM, which is composed of collagen fibers, fibronectin, elastin, and matricellular proteins (Table 2). The deposition of ECM proteins in the LV or vasculature can lead to structural and functional changes due to both direct effects of the ECM proteins as well as indirect effects of ECM proteolytic processing. For example, fragments generated due to MMP-9 cleavage of collagen have suppressive effects on pathological angiogenesis, as well as stimulatory effects on both mRNA and protein levels of MMP-9 (27, 39).

Several ECM proteins are proteolytically processed by MMP-9, including collagen, fibronectin, and laminin. ECM fragments are known to express bioactive properties and regulate cardiac and vascular remodeling (117). Laminin is a well known MMP-9 substrate, and its levels negatively correlate with increased levels of MMP-9 (46). Laminin fragments support adhesion and differentiation of stem cells in vitro, playing an important role in wound healing (77). Laminins increase early post-MI and are associated with decreased cardiac rupture. They may impair macrophage infiltration and delay wound healing (73). In patients with hypertension, fibronectin levels are increased in the tunica media of the arteries, whereas laminin levels remain unchanged (103). In post-MI conditions, the levels of fibronectin and laminin are increased (143). Fibronectin fragments generated by MMP-9 cleavage act as chemoattractants for a variety of cell types involved in infarct healing and trigger a feedback mechanism to induce fibronectin expression (143).

Thrombospondin and tenascin-C regulate cell-matrix interactions. Thrombospondin-1 increases 7–28 days after ischemia-reperfusion and is known to inhibit MMP-9 activity (30). The higher level of thrombospondin-1 in the LV of MMP-9-null mice post-MI may explain the beneficial effects of MMP-9 deletion on angiogenesis (68). Tenascin-C plays an important role in the proliferative phase of the infarct healing. Tenascin-C often co-localizes with the MMP-9 site of active remodeling, and the deletion of MMP-9 was reported to increase the production of this matricellular protein (143).

Table 3. *Effects of intracardiac-injected substances on myocardial structure and function after injury*

Substance Injected	Effects
ECM-derived Hep I	Promotes cell attachment, migration, and proliferation; induces Erk1/2 activation; promotes angiogenesis and arteriogenesis
ECM-derived Hep III	Promotes cell attachment, migration, and proliferation; induces Erk1/2 activation; promotes angiogenesis and arteriogenesis; prevents worsening of LV function; interacts with other Hep III peptides and ECM proteins; forms polymer-like matrix
ECM-derived RDG	Promotes cell attachment, migration, and proliferation; induces Erk1/2 activation; promotes angiogenesis and arteriogenesis
ECM-derived FC/HV	Adherent cells noticeable within 24 h; induces Erk1/2 activation
Injection of decellularized matrix	Thickens the LV infarcted wall; prevents LV systolic dysfunction; improves EF
Fibrin	Preserves infarcted wall thickness and cardiac function
Tannic acid	Prevents collagen degradation via cross-linking; inhibits MMP-9 activity

Effects of intracardiac-injected substances on myocardial structure and function after injury (14, 60).

Tenascin-C weakens the adhesive interactions involving cardiomyocytes and contributes to reverse cardiac remodeling (30).

Galectin-3 is a carbohydrate-binding protein that contains a collagen-like domain susceptible for direct and rapid cleavage by MMP-9 (84). Galectin-3 serves as a diagnostic biomarker for MMP-9 activity. Galectin-3 is capable of binding to laminin, fibronectin, and collagen IV and is expressed in foam cells and macrophages (137). Higher concentrations of galectin-3 and increased MMP-9 activity are associated with cardiac fibrosis and increased risk for HF and mortality (45).

MMP-9 is known to process a number of inflammatory chemokines through the proteolysis. MMP-9 processes CXCL5 at the NH_2 terminus and increases its chemotactic activity twofold (123). MMP-9 increases the chemotactic properties of CXCl8 (122). MMP-9 inactivates CXCL1, CXCL4, CXCL5, CXCL7, and CXCL12 (75, 122, 123). CXCL6 has been shown to be cleaved by MMP-9, but a change in its biological properties has not been reported (123). Evaluated properties of the described chemokines have been performed ex vivo in soluble proteins; however, in vivo these chemokines are immobilized on the ECM or cell surface by binding to glycosaminoglycans via positively charged domains (124).

MMP-9 processes a number of cytokines, including TNF-α, IL-1β, and TGF-β. MMP-9 was shown to release active TNF-α from the cell surface via proteolysis, which results in the production of a biologically active mature form (33). IL-1β is produced in an inactive form and is cleaved mainly by the caspase-1 to its active form by removing the NH_2 terminus of the cytokine. MMP-9 both cleaves the inactive form of IL-1β to its active state and degrades its active form to decrease its biological activity (110). TGF-β is an anti-inflammatory cytokine and is released in the extracellular space in the latent form. A latent form of TGF-β is cross-linked to the ECM, and its maturation is associated with several mechanisms, including proteolysis. MMP-9 is capable of cleaving the latent pro-form of TGF-β to its active state (140). Another possible mechanism of TGF-β activation is through the degradation of the decorin, a small collagen-associated proteoglycan. Decorin serves as a depot for TGF-β in ECM, and its degradation by MMP-9 leads to a proteolysis of the cytokine and its activation (48).

Recent advances into the identification of ECM proteins in cardiovascular diseases suggest that MMP-9 has a wide array of potential substrates (3). However, the specificity and the functions of peptides generated from these substrates have yet to be evaluated.

Translational Applications

The initial approach to translating MMP-9 research concentrated on inhibiting the enzyme. Among all the MMP inhibitors designed and generated, only nonspecific doxycycline (periostat) has been approved by the Food and Drug Administration (94). Selective inhibitors to MMP-9 have been designed based on motifs of the active site (HWGF, CRRH-WGFEFC, and CTTHWGFTLC). These peptides are susceptible to active proteolysis in vivo, which limits their suitability for experimental clinical settings (44, 57). A few selective MMP-9 inhibitors have been generated and successfully tested in the models of cardiovascular disease. In the cardiac injury setting, salvianolic acid, a selective MMP-9 inhibitor, prevented cardiac remodeling in spontaneously hypertensive rats (49). However, MMP-9 possesses not only deleterious effects but also beneficial effects depending on the time of the progression of the cardiovascular disease. Inhibition of MMP-9 in the acute stages of the cardiovascular disease may provide favorable outcomes, but MMP-9 inhibition in the later stages of the disease may alter the compensatory remodeling and contribute to a progression into HF.

A number of medications used in the treatment of patients with atherosclerosis, hypertension, and MI overlap in their efficacy to inhibit the production and activity of MMP-9. Medications targeting

the renin-angiotensin-aldosterone system include ACE inhibitors, angiotensin receptor blockers, and aldosterone antagonists, and inhibit MMP-9 activities in animals and patients with MI and/or HF (66, 88). The aldosterone receptor-blocker eplerenone decreased MMP-9 activity in dogs with HF (98). ACE inhibitors reduced MMP-9 activity by directly binding to the MMP-9 active site (135). Beta blockers decrease MMP-9 expression and activity. Carvedilol was reported to reduce plasma levels of MMP-9 in patients with idiopathic cardiomyopathy and improved LV remodeling in a mouse model of acute myocarditis (91). Statins, pravastatin in particular, reduce serum levels of MMP-9 in post-MI patients (139).

Conclusions and Future Directions

Although selective MMP-9 inhibition is still a concept that is under development, targeting other mechanisms that stimulate MMP-9 activation may be beneficial. Administration of an endothelin-1 receptor blocker 3 days after MI reduced MMP-9 levels and activity, decreased TIMP-1 levels, and prevented LV dilation (95). Targeting serine proteases and the plasminogen system is another approach to regulate MMP-9 activity. Mice deficient in uPa or treated with plasminogen activator inhibitors showed protection against cardiac rupture that was mediated through reduced MMP-9 activation (42, 43). Other serine proteases, including serine elastase, trypsin, and cathepsin G, also induce MMP-9 activity and are capable of destroying the inhibitory activity of TIMPs. Inhibition of serine elastase was reported to reduce neutrophil accumulation into the ischemic myocardium and suppress MMP-9 activity (19, 141). There are, therefore, multiple indirect mechanisms of inhibiting MMP-9 function.

A better understanding of ECM fragments and MMP-9 activity may provide new opportunities to regulate inflammation by binding their chemotactic properties and attenuating cardiac remodeling. (76). Several studies have already reported the beneficial use of intracardiac injection of ECM-derived collagen and fibronectin in the post-MI setting (Table 3) (18, 76). Finding new MMP-9 substrates is another promising approach. Barallobre-Barreiro and colleagues identified more than 100 ECM proteins expressed in the focal region of the injured heart in ischemic cardiomyopathy patients, suggesting that there may be more substrates for MMP-9 activity than expected (3).

Another possible approach is targeting ECM components directly at the time of injury. For example, intracardiac injections of tannic acid and fibrin have been shown to stabilize the ECM and preserve cardiac structure and function (14, 145). The results obtained to date provide new perspectives to the translational applications of both diagnostic and therapeutic strategies related to MMP-9 activity.

In this review, we summarized and discussed the roles of MMP-9 in normal development and in multiple cardiovascular diseases, including hypertension, atherosclerosis, MI, and HF. The ability of currently used medications in cardiac disease to directly or indirectly affect the production and activity of MMP-9 suggests that this metalloproteinase plays a key role in many of the molecular mechanisms underlying these pathophysiological conditions. However, selective inhibition of MMP-9 remains an open issue. Future successful in vivo studies using selective MMP-9 inhibitors may provide new insights and perspective to intervene on ECM remodeling in humans. ∎

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