Myocardial extra-cellular matrix and its regulation by metalloproteinases and their inhibitors

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Summary
Cardiovascular disease poses a major health care burden in the Western world. Following myocardial injuries, ventricular remodelling and dysfunction ensue, which can eventually culminate in heart failure. An important event in left ventricular (LV) remodelling is alteration of the extracellular matrix (ECM) integrity, the structural network that interconnects the myocardial components. The critical role of ECM remodelling in cardiac dilation and heart failure was recognized more than a decade ago, and the molecular factors responsible for this process are now being explored. Abnormal ECM turnover is primarily brought about by an imbalance in the activity of matrix metalloproteinases (MMPs) that degrade ECM components, and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs). Here we provide an overview of composition of the cardiac ECM, and alterations in ECM regulatory proteins, MMPs and TIMPs, in human heart disease. We also discuss the role of TIMPs, MMPs, and a disintegrin and metalloproteinase (ADAMs) enzymes in cardiac development and function as learned through genetically altered mouse models.

Keywords
Extracellular matrix, metalloproteinases, tissue inhibitor of metalloproteinases, heart disease, genetic mouse models

Cardiac extracellular matrix
Optimal cardiac function requires the orchestration of multiple mechanical and biochemical factors within the myocardial microenvironment. The extracellular matrix (ECM) proteins provide the structural foundation for the myocardium, while soluble, matrix-bound, and cell surface proteins deliver molecular cues to the signaling pathways for myocyte survival and contraction. ECM structure in mammalian heart is comparable among species. It provides a scaffold for myocytes, fibroblasts, endothelial cells and the vasculature to align and build a network. This results in the formation of a muscle of extraordinary tensile strength, essential for efficient systolic and diastolic function. It is now recognized that not only disturbances in the contractile apparatus of the cardiomyocytes, but alterations in interstitial architecture of the myocardium also play a central role in the pathogenesis of cardiac failure (1).

Precise myocardial ECM composition of fibrillar collagens, basement membrane components, and proteoglycans is essential for proper cardiac geometry and function. Collagen types I and III are the predominant interstitial collagens in the myocardium that generate structural integrity for the adjoining cardiomyocytes, providing the means by which myocyte shortening is translated into overall ventricular pump function. The precise ratio of these collagens is critical in determining myocardial stiffness. The network of collagen fibers exists at three levels: endomysium, epimysium and perimysium. Endomysium surrounds individual muscle fibers, while the epimysium network surrounds a group of muscle fibers. The perimysium consists of thick, spiral-shaped bundles of collagen that connect epimysial and endomysial networks. Collagenous “struts” connect adjacent myocytes, and myocytes to capillary endothelium to attain capillary stability in the presence of high intraventricular pressure. Basement membrane components include laminin, entactin, fibronectin, collagen type IV, and fibrillin. The noncollagenous glycoproteins, fibronectin and laminin, are important for cell adhesion and cell-cell interaction. Proteoglycans include chondroitin sulfate, dermatan sulfate, and heparan sulfate. Membrane-associated proteoglycans are predominantly heparan-sulfate substituted, which can be either transmembrane or glycosylphos-
phatidylinositol anchored. Syndecans are transmembrane proteoglycans, with a family of four members, syndecan-1 to -4 (2). Studies on mRNA abundance have shown that syndecan-1 is undetectable in the mouse heart, while syndecan-2 and syndecan-3 are present in intermediate amounts, and syndecan-4 in low amounts (3). A role for syndecan-4 in adhesion and signal transduction has been suggested in neonatal rat cardiomyocytes (4).

Vasculature is an important component of the cardiac tissue. The main protein constituents of ECM in vessels are collagen and elastin, with elastin being the most abundant protein in large arteries that are continually subjected to pulsatile pressure generated by cardiac contraction (5). In vascular diseases, degradation and fragmentation of collagen and elastin fibers are increased (6). Vascular ECM remodelling has been reviewed extensively (6–8). In this review, we will focus on cardiac ECM in health and disease.

In the myocardium, ECM is linked to cellular cytoskeleton by integrins. These transmembrane heterodimers provide a physical connection between the cell surface and the surrounding ECM proteins (9, 10). An integrin-mediated insertion into the basal lamina localized to the Z band of the sarcomere forms direct connections between adjacent cardiomyocytes. Integrins a1b1, a3b1 and a5b1 are expressed on cardiac myocytes, while b1D is the cardiac-specific sub-isoform (11, 12). An intriguing role of integrins in the heart is their ability to serve as mechanotransducers during normal heart development and in response to physiologic and pathophysiologic signals (13). Integrins are also involved in bi-directional signal transduction (reviewed in (14)). Focal adhesion kinase (FAK) is the key cytoplasmic tyrosine kinase that transmits integrin-mediated signals in several cell types (15, 16). A significant role for integrins and FAK has been proposed in cardiac disease since a-adrenergic stimulation increases both b1D level and FAK phosphorylation, and over-expression of b1D or FAK triggers hypertrophy in cultured rat neonatal myocytes (17). Other evidence linking these factors to cardiac disease are the significant increases in FAK tyrosine phosphorylation, activation of PKB/Akt (through phoshatidylinositol 3-kinase, PI3K) and ERK1/2 early after induction of pressure overload in the rat (18). Thus, ECM serves a structural function and is also linked to signal transduction pathways.

**Cardiac ECM undergoes remodelling in heart disease**

Aberrant cardiac remodelling in disease is accompanied by a change in mass, volume, and shape of the ventricles. This can be brought about by a number of factors including myocardial infarction, hypertension, valvular disease, familial hypertrophic cardiomyopathy and dilated cardiomyopathy (19). Chronic heart failure, irrespective of its cause, is characterized by an overall imbalance of ECM turnover with myocardial collagen accumulation, collagen fibril disruption, myocyte loss, and altered spatial orientation of cells and intracellular components (19, 20). This maladaptive remodelling contributes to diminished systolic performance, decreased compliance, and diastolic dysfunction in failing human heart (20). Often in heart disease the problem arises from altered arrangement and reduced cross-linking between collagen fibers rather than a reduction in total collagen content. For instance, in patients with idiopathic dilated cardiomyopathy, an excess proportion of collagen type III and a reduction in the number of thick collagen fibers connecting adjoining muscle fiber bundles are seen. Despite the significant increase in collagen production, the replacement collagen is poorly cross-linked (21). This compromises the supportive scaffolding leading to cell slippage, LV dilation and diminished diastolic compliance (22). The turnover rate for collagen is remarkably slower than non-collagen proteins (>10 times slower synthesis (23), >10 times longer half-life [24]), thus making the myocardium vulnerable to aberrant remodelling in conditions associated with increased ECM degradation.

ECM integrity is maintained by a balance in the activity of matrix metalloproteinases (MMPs), a family of enzymes that degrade ECM proteins, and their tissue inhibitors, TIMPs. Dysregulation in this balance can be brought about by a number of factors (reviewed in (25)). In cardiac fibroblasts, the inflammatory cytokines such as interleukin-1β (IL1β), interleukin-6 (IL6) and tumor necrosis factor-a (TNFa) decrease collagen synthesis, increase MMP expression and decrease TIMP expression (26, 27). Inflammatory cytokines are tightly linked to tissue stress and their production is induced following biological stimuli (28, 29) such as pressure overload and myocardial injury (30). Transforming growth factor-β (TGFβ) is an anti-inflammatory cytokine and a potent stimulator of collagen synthesis. It mediates collagen accumulation through increasing transcription (31), stabilizing procollagen mRNA (32), and decreasing collagen degradation via enhanced TIMP or reduced MMP expression (33). Sustained production of TGFβ underlies the development of myocardial fibrosis (34). Not only these cytokines impact ECM integrity, but the cell surface processing of inflammatory cytokines or activation of TGFβ from its latent ECM-bound form itself is facilitated through ADAMs and MMPs (reviewed in (35, 36)). This can create a complex feedback loop whereby high ADAM/MMP activity leads to increased cytokine bioactivity further contributing to ECM remodelling. Thus, the extent of ECM remodelling depends partly on a balance between pro-inflammatory and anti-inflammatory cytokines, which can be differentially activated depending on the type of myocardial insult or the stage of disease progression. The parallel temporal involvement of these two groups of cytokines, and their precise relationship with the metalloproteinase axis is not well defined in cardiac disease. A detailed understanding of factors that regulate myocardial matrix remodelling, including pro-inflammatory and anti-inflammatory cytokines, metalloproteinases, and TIMPs, is necessary for gaining new insights into managing heart disease.

**Metalloproteinases**

Following the sequencing of human and mouse genomes, a phylogenetic tree called a protease wheel has been generated that illustrates the five major classes of proteases (reviewed in (37)). Metalloproteinases have a zinc binding site that is essential for their activity, and are classified according to the primary structure of their catalytic sites into glu zincin, metzincin, inuzincin, carboxypeptidase, and DD carboxypeptidase subgroups. The metzincin subgroup is further divided into serralysins, astacins, matrixins and adamalysins (reviewed in (38)). The matrixins in-
clude the MMPs, while ADAMs belong to the adamalysin subgroup.

**MMPs: the classical ECM degrading metalloproteinases**

MMPs are Ca\(^{2+}\) - and Zn\(^{2+}\)-dependent proteases that are primarily synthesized as inactive zymogens (Pro-MMPs), requiring activation by the removal of an amino-terminal propeptide domain either by autoproteolysis or processing by another MMP or serine protease. The mechanism of MMP-2 activation has been widely studied. Its activation at the cell surface occurs through the trimolecular complex involving TIMP-2 and a transmembrane MMP such as MMP-14 (MT1-MMP) (39). To date, 24 MMPs have been identified in vertebrates (40) and classified in a number of ways; numerically, according to ECM substrate specificity, or based on shared functional domains. The currently known MMPs and their common names are listed in table 1.

MMPs can collectively process all components of the ECM (37, 41). The classes of MMPs suggested to have particular relevance to myocardial remodelling are the collagenases MMP-1 and -13, the stromelysin MMP-3, the gelatinases MMP-2 and -9, and the membrane-type MMP, MMP-14 (MT1-MMP) (42). MMP-1 degrades collagen types I, II, III and basement membrane proteins, MMP-13 can process collagen types I, II and III, and MMP-14 can cleave fibronectin, gelatin, laminin-1 (43) and fibrillar collagen type I (44, 45). MMP-2 and MMP-9 have been shown to bind and process fragments of a number of collagens, such as type I, IV and V, while MMP-2 can also cleave collagen type III fragments (46, 47). Rodents lack the MMP-1 gene, and it is believed that MMP-13, and recently identified MMP-1a (mColA) and MMP-1b (mColB) provide the necessary collagenolytic activity (48). Notably, MMP-1a and MMP-1b are not expressed in mouse heart (49). Beyond the ECM proteins, MMPs are now recognized to have non-ECM substrates including a number of growth factors and cytokines (35, 36), adding to the levels at which MMPs influence tissue remodelling.

Localization of a protease is an important determinant in its actions. Membrane type MMPs (MT-MMPs) are covalently linked to the cell membrane (41). Some of the secreted MMPs also localize to the cell surface by binding to an integrin (50), cell surface hyaluronan receptor CD44 (51), through interaction with cell surface associated heparan sulfate proteoglycans, collagen type IV, or the extracellular matrix metalloproteinase inducer (emmprin) (47). In the myocardium, MMPs are expressed by fibroblasts (52) and cardiomyocytes (53), and primarily function extracellularly (47). Interestingly, recent studies in cardiomyocytes suggest the intracellular presence of MMP-2, where it colocalizes with troponin I and α-actinin along the sarcomere Z-lines (53, 54). Such intracellular localization of MMP-2 has been suggested to affect intracellular contractile filaments. Whether MMP-2, whose activation depends on the trimolecular complex at the cell surface, can also be activated intracellularly remains unclear.

### Table 1: List of MMPs in vertebrates and their common names.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>MMP</th>
<th>Other names</th>
</tr>
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<tbody>
<tr>
<td>Collagenase 1</td>
<td>MMP-1</td>
<td>Interstitial collagenase, mColA, mColB (mouse)</td>
</tr>
<tr>
<td>Collagenase 2</td>
<td>MMP-8</td>
<td>Neutrophil collagenase</td>
</tr>
<tr>
<td>Collagenase 3</td>
<td>MMP-13</td>
<td></td>
</tr>
<tr>
<td>Collagenase 4</td>
<td>MMP-18</td>
<td></td>
</tr>
<tr>
<td>Gelatinase A</td>
<td>MMP-2</td>
<td>72-kDa gelatinase</td>
</tr>
<tr>
<td>Gelatinase B</td>
<td>MMP-9</td>
<td>92-kDa gelatinase</td>
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<tr>
<td>Metrilysin</td>
<td>MMP-7</td>
<td></td>
</tr>
<tr>
<td>Metrilysin 2</td>
<td>MMP-26</td>
<td>Endometase</td>
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<tr>
<td>Stromelysin 1</td>
<td>MMP-3</td>
<td></td>
</tr>
<tr>
<td>Stromelysin 2</td>
<td>MMP-10</td>
<td></td>
</tr>
<tr>
<td>Stromelysin 3</td>
<td>MMP-11</td>
<td></td>
</tr>
<tr>
<td>Metalloelastase</td>
<td>MMP-12</td>
<td>Macrophage elastase</td>
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<tr>
<td>MT1-MMP</td>
<td>MMP-14</td>
<td></td>
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<tr>
<td>MT2-MMP</td>
<td>MMP-15</td>
<td></td>
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<tr>
<td>MT3-MMP</td>
<td>MMP-16</td>
<td></td>
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<tr>
<td>MT4-MMP</td>
<td>MMP-17</td>
<td></td>
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<tr>
<td>MT5-MMP</td>
<td>MMP-24</td>
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<tr>
<td>MT6-MMP</td>
<td>MMP-25</td>
<td></td>
</tr>
<tr>
<td>Enamelysin</td>
<td>MMP-20M</td>
<td></td>
</tr>
<tr>
<td>Eplysin</td>
<td>MMP-28</td>
<td></td>
</tr>
<tr>
<td>RASI-1</td>
<td>MMP-19</td>
<td></td>
</tr>
<tr>
<td>CMMP (Chicken)</td>
<td>MMP-22</td>
<td></td>
</tr>
</tbody>
</table>

**ADAMs: metalloproteinases that release cell-surface molecules**

ADAMs are similar to MMPs in their metalloproteinase domain, and also contain a unique integrin-binding disintegrin domain. To date, 34 ADAMs have been described in a variety of species with 19 in humans (38). Not all ADAMs possess a functional metalloproteinase domain. ADAMs are synthesized in the rough endoplasmic reticulum and mature in the late Golgi compartment following removal of the pro-domain (55–57). These membrane-anchored enzymes bring about the shedding of numerous cell surface and matrix-bound proteins and are thus also called sheddases (58, 59). Among the cell surface molecules processed by ADAMs are growth factors, including heparin-binding epidermal growth factor (HB-EGF) and transforming growth factor-α (TGFα), and cytokines, such as TNFα, IL-1 and IL-6 (58, 60, 61). These molecules are known to influence myocardial remodelling by inducing hypertrophy and/or apoptosis (62–64). ADAM-12 has been shown to play an integral role in the hypertrophic response upon stimulation of G-protein coupled receptors (GPCRs), through cleavage of membrane-bound HB-EGF and subsequent transactivation of the EGF receptor (EGFR) (65). ADAM-10 and ADAM-17 (TNF-α converting enzyme,
TACE) also shed HB-EGF (66, 67). The latter further releases membrane-bound TNFα and TGFα (68, 69).

Thus, the diversity of substrates now linked to metalloprotei-

nase activity show that the role of MMPs and ADAMs in the

myocardium extends beyond ECM degradation to their involve-

ment in cardiac structure, function and response to injury by

regulating the release of ligands critical to cardiomyocyte hyper-

trophy and apoptosis. It is important to identify the physiological

functions of specific MMPs and ADAMs in myocardial develop-

ment and in response to myocardial diseases, to develop a

basis for effective therapeutic interventions.

**Metalloproteinase inhibitors**

Activity of MMPs is controlled by a series of endogenous inhibi-

tors. Some of these are general protease inhibitors such as α2-

macroglobulin that blocks MMP activity in plasma and tissue

fluids (70). Alpha 2-macroglobulin irreversibly clears MMPs by

forming a complex that is subsequently removed by scavenger

receptor-mediated endocytosis (47). TIMPs are specific MMP

inhibitors in the tissue compartment and have a more complex

role. They reversibly inhibit activated MMPs through binding in

a 1:1 stoichiometry. Additionally, specific TIMPs are involved in

cell surface activation of specific MMPs. MMP activity may also

be blocked by a number of other proteins that contain sequences

with similarities to the N-terminal domain of TIMPs. Examples of

these include the procollagen C-terminal proteinase enhancer

(71), the NC1 domain of type IV collagen (72), and the tissue

factor pathway inhibitor-2 (73). Finally, reversion-inducing cy-

teine-rich protein with kazal motif (RECK) is a cell surface pro-

tein with MMP inhibitory capacity (74). Among these inhibitors,

TIMPs have been most extensively studied in cardiovascular pa-

thology.

There are four TIMPs in vertebrates. TIMP-1 and -3 are tran-

scriptionally induced by growth factors and cytokines, while

TIMP-2 and -4 are mostly constitutively expressed. TIMP-1,

TIMP-2 and TIMP-4 are present in soluble form, while TIMP-3

binds to the ECM via heparan sulfate proteoglycans within the

ECM (75, 76). TIMPs efficiently inhibit MMPs albeit with dif-

ferent specificity and affinity (77–79). In addition to inhibiting a

broad spectrum of MMPs, TIMP-3 is also an effective inhibitor of

ADAMs (e.g., ADAM-12 and ADAM-17), as well as ADAMs

with thrombospondin domain (e.g., ADAMTS-4 and

ADAMTS-5) (80, 81). TIMP-1 reportedly inhibits ADAM-10

(82) and ADAMTS-1 (83).

The number of MMPs far exceeds the number of TIMPs. This

may be due to the broad inhibitory capacity of TIMPs against

MMPs, as suggested by biochemical studies. Moreover, the in-

hibitory function of a TIMP is only required as a final control of

an activated MMP. Most MMPs are not constitutively expressed

or activated. Thus, tightly regulated transcription of MMPs, their

activation processes, in addition to inhibition of activated MMPs

provide sufficient means of keeping MMP activity in check.

Further testing in physiological systems, including the cardiac

models, is required to reveal whether specific TIMPs favour in-

hibition of specific MMPs *in vivo*.

**Cardiac expression of metalloproteinases and their inhibitors in mouse**

A recent study by Edwards’ group (49) has shown that in adult

mouse heart, MMP-2 and MMP-15 (MT2-MMP) are most

highly expressed, MMP-11, -13, -19, -23, -24 and -28 are highly

expressed, while MMP-3, -8, -9 and -12 are moderately express-
ed. MMP-1a, MMP-1b, MMP-7, MMP-10, and MMP-20 are not

detectable in this organ. MMP-14 (MT1-MMP) is expressed at

very high levels in the embryonic heart and up to 7 days post-par-
tum, but its levels somewhat decline in the adult. Among

ADAMs, ADAM-15, -17, and -19 are most highly expressed in

adult murine heart, ADAM-10 and -12 are highly expressed,

whereas ADAM-28 is undetectable (49). This group also re-

ported that TIMP-2 and TIMP-3 are most abundant in the adult

murine heart followed by TIMP-4 and TIMP-1, with all TIMPs

showing a transient increase within one week of birth (49, 84).

Among TIMPs, TIMP-4 shows a more restricted tissue express-

sion pattern with high levels in the heart (49, 84, 85), al-

though TIMP-3 has the highest mRNA copy number in this

organ (84). Such relative abundance of MMPs, ADAMs and

TIMPs in the myocardium suggests specific roles for these mol-

ecules in heart structure and function. Moreover, these data form

a baseline for further dissection of the physiological role of each

gene in cardiac development and in disease progression through

biochemical and genetic manipulation strategies.

**TIMPs and MMPs in human heart disease**

The normal balance between activities of MMPs and TIMPs is

lost in cardiac pathologies. Figure 1 summarizes the reported

changes in MMPs and TIMPs at the mRNA and protein levels in

different types of heart disease in human. In patients with

ischemic cardiomyopathy or idiopathic dilated cardiomyopathy

(DCM), reduced mRNA levels of *timp-1* and *timp-3* and protein

levels of TIMP-1, TIMP-3 and TIMP-4, but no change in TIMP-2

levels were observed (86). These alterations in TIMP levels were

associated with a significant increase in MMP-1, MMP-2,

MMP-9, MMP-13 and MMP-14 (MT1-MMP) protein levels

(86–91). Thus, it appears that DCM is generally associated with
decreased TIMP and increased MMP levels. However, some dis-
crepancies exist in the reported studies. For example, TIMP-1

levels have been reported to be either reduced (87) or increased

![Figure 1: Alterations in MMP and TIMP levels in human heart disease. Italic lower case letters depict mRNA levels, capital letters indicate protein levels, ↑ and ↔ represent increase, decrease and no change, respectively. * denotes circulating plasma levels.](downloaded_from_the_web.png)
(89) in DCM patients. These differences could arise from the fact that the former group studied patients at undefined stages of DCM, whereas the latter group examined only end-stage DCM LVs. Moreover, these end-stage DCM patients were chronically treated with angiotensin-converting enzyme (ACE) inhibitors, which may have affected myocardial TIMP or MMP expression (92). Right ventricular biopsies in DCM patients showed a negative correlation between MMP-2 and TIMP-2 levels and the LV ejection fraction (93).

In hypertrophic cardiomyopathy patients, both MMP-2 and TIMP-2 were found elevated with systolic dysfunction but not in those with preserved systolic function, whereas TIMP-1 was elevated in both groups, and MMP-3 and MMP-9 were not altered in either group (94). In patients with progressive heart failure, TIMP-1 and MMP-1 levels were significantly elevated compared to donor hearts (95). In rheumatic heart disease, the severity of cardiac dysfunction directly correlated with increased MMP-3 mRNA and inversely with that of TIMP-1 (96). Further, a correlation between TIMP-1 levels and fibrosis has been shown in hypertensive patients (97, 98). Timms et al. (97) suggested that the increased inhibitory effects of TIMP-1 on collagen degradation was responsible for the elevated tissue collagen III levels in the heart and vessels of these patients, whereas Lindsay et al. (98) found evidence of increased synthesis as well as increased inhibition of collagen degradation resulting in fibrosis. Measurements of MMP and TIMP levels in plasma have also been used as estimates of heart disease progression. Plasma from patients with congestive heart failure (CHF) showed increased MMP-9, decreased MMP-8 and no change in MMP-2 levels. In addition, a 3-fold increase in MMP-9:TIMP-1 ratio and a 16-fold increase in MMP-9:TIMP-2 ratio were observed in these patients (99). Another study reported higher circulating MMP-2 in patients with severe CHF than in those with mild CHF, while both groups were higher than control patients (100).

Altogether these studies indicate that although maladaptive LV remodelling in cardiac disease is generally associated with enhanced MMP and reduced TIMP activities, this pattern is not held universally and varies with the type and stage of disease. As our understanding of MMPs and TIMPs grows, it may be feasible to establish specific MMP/TIMP profiles as biomarkers to assess the degree of cardiac pathology. New developments, such as those that measure MMP or TIMP levels or activity at the tissue level, for instance by microdialysis technique, will add significantly towards our understanding of the tissue MMP/TIMP imbalance in relation with disease progression.

**Genetic models of metalloproteinases and inhibitors related to heart disease**

**Role of TIMPs in cardiac development and function**

Genetic manipulation of different MMPs or TIMPs in mice has provided insights into their roles in cardiovascular development and in progression of cardiac disease. These mouse models are listed in table 2 which focuses on their cardiac-related phenotypes. Partial loss of MMP inhibitory control through *timp-1* gene deletion causes LV dilation in mice at four months of age, although LV systolic pressure and ejection fraction are preserved, and no myocyte hypertrophy is detected (101). These mice also exhibit adverse LV remodelling, such as increased dilation and hypertrophy and reduced cardiac function, after myocardial infarction (MI) (102). *Timp-2* null mice show reduced MMP-2 activity, but are otherwise normal and healthy (103). These mice have not been subjected to experimental cardiovascular disease models to date. We have recently reported that ablation of *timp-3* leads to cardiomyocyte hypertrophy, DCM and contractile dysfunction in 21-month old mice. Absence of *timp-3* in these mice results in disruption of interstitial collagen fibers with elevated MMP-9 and TNFα activities (104). *Timp-3*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cardiac phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>timp-1</em></td>
<td>LV dilation at 4 months of age, preserved systolic pressure and ejection fraction (101) Amplified adverse LV remodeling after MI (102)</td>
</tr>
<tr>
<td><em>timp-2</em></td>
<td>Reduced MMP2 activity, otherwise healthy (103) No report on cardiac phenotype</td>
</tr>
<tr>
<td><em>timp-3</em></td>
<td>Dilated cardiomyopathy at 21 months of age (104) Enlarged pulmonary airspace at 18 months of age (105) Accelerated apoptosis in mammary epithelial cells (106)</td>
</tr>
<tr>
<td><em>timp-4</em> (cardiac-specific overexpression)</td>
<td>Decreased heart/body weight ratio, increased fibrosis and apoptosis, diastolic dysfunction (Vuoño's lab, personal communication).</td>
</tr>
<tr>
<td><em>reck</em></td>
<td>Fetal death due to poor collagen formation and vascular defects (74)</td>
</tr>
<tr>
<td><em>mmp-1</em> (interstitial collagenase)</td>
<td>Myocyte hypertrophy, increased cardiac collagen at 6 months of age. Systolic and diastolic dysfunction at 12 months of age (107)</td>
</tr>
<tr>
<td><em>mmp-2</em> (gelatinase A)</td>
<td>Reduced LV dilation, improved fractional shortening, lower incidence of LV rupture after MI (108)</td>
</tr>
<tr>
<td><em>mmp-9</em> (gelatinase B)</td>
<td>Attenuated LV dilation following MI (109) Protected against ischemia-induced myocardial injury (110)</td>
</tr>
<tr>
<td><em>mt1-mmp</em> (MMP-14)</td>
<td>Effects in skeletal development, dwarfism, osteopenia, arthritis and connective tissue disease (111, 112) No report on cardiac function</td>
</tr>
<tr>
<td><em>adam-9</em> (meltrin γ)</td>
<td>Viable, healthy, fertile (113)</td>
</tr>
<tr>
<td><em>adam-10</em></td>
<td>Developmental defects in cardiovascular and central nervous systems Death at day 9.5 of embryogenesis (113)</td>
</tr>
<tr>
<td><em>adam-12</em> (meltrin α)</td>
<td>30% mortality 1 week postnatal, impaired adipogenesis and myogenesis, otherwise normal and viable (114) No report on cardiac function</td>
</tr>
<tr>
<td><em>adam-17</em> (TACE)</td>
<td>Perinatal death. Enlarged fetal hearts (116) Abnormal lung development (117)</td>
</tr>
<tr>
<td><em>adam-19</em> (meltrin β)</td>
<td>Perinatal death, Ventricular septal defect, valvular stenosis and abnormal cardiac vasculature (123, 124)</td>
</tr>
</tbody>
</table>
null mice also develop enlarged pulmonary airspace at 18 months of age (105), and display accelerated apoptosis in mammary epithelial cells (106). Cardiac specific overexpression of TIMP-4 results in reduced heart to body weight ratio, increased fibrosis and apoptosis, and spontaneous LV diastolic dysfunction (personal communication with Vuorio lab, Turku, Finland). RECK downregulation has been implicated in tumour angiogenesis, and mice lacking a functional RECK gene die around E10.5 with defects in collagen fibrils, the basal lamina, and vascular development (74). The role of RECK in cardiac development and function will require generation of cardiac-specific deletion of this gene. Given the general abundance of all TIMPs in the developing and the adult murine heart, more remains to be learned through the use of these mutants in specific cardiac challenges.

**Role of MMPs in heart structure and function**

Cardiac-restricted overexpression of human MMP-1 results in ventricular hypertrophy and hypercontractility in young mice and ventricular dilation and failure in 1-year-old mice (107). Mice lacking mmp-2 exhibit reduced LV dilation, improved fractional shortening, and lower incidence of LV rupture after myocardial infarction (108), while those lacking the other gelatinase, mmp-9, have reductions in LV remodelling, LV enlargement and collagen accumulation after this challenge (109). Mmp-9−/−mice are also protected against in vivo no-flow ischemia-reperfusion-induced myocardial injury (110). Mice deficient in mmp-14 (mt1-mmp) exhibit defects in skeletal development and angiogenesis (111), severe bone and connective tissue abnormalities and disease (112). No report on cardiovascular function in these mice currently exists. These studies highlight the degradative capacity of MMP-1, -2 and -9 in the heart, whereas mmp-14 (mt1-mmp) function in heart remains less well understood due to the developmental and connective tissue disorders at an early age in the absence of this protein. The role of mmp-14 (mt1-mmp) in cardiac development and in response to heart disease can be fully explored by generating cardiac-specific mmp-14 (mt1-mmp) deficient mice. Despite the evolving information on specific MMPs in cardiovascular disease, the role of a large number of MMPs that are highly expressed in the heart, such as MMP-8, -11, -12, -13, -15, -19, -23, and -24 remains to be determined.

**ADAMs and their role in cardiac development**

A number of ADAMs are expressed in the heart and are shown to be involved in cardiovascular development. Mice lacking ADAM-10 die at day 9.5 of embryogenesis due to defects in cardiovascular and central nervous systems (113). ADAM-12/meltrin α has been suggested to mediate cardiac hypertrophy through shedding of HB-EGF (65). Ablation of this ADAM results in ~30% mortality at birth, however the viable homozygous mice are normal and fertile with impaired adipogenesis and myogenesis (114). HB-EGF shedding by 12-O-tetradecanoyl phorbol-13-acetate (TPA) is reduced in adam-12−/−fibroblasts (114). No cardiovascular defect has yet been reported in these mice. However, mice lacking HB-EGF develop severe heart failure with gross ventricular dilation, enlarged cardiac valves and diminished cardiac function with 50% mortality in the first postnatal week (115). ADAM-10 and ADAM-17 also shed HB-EGF (66, 67) which could explain the lack of cardiac defects in ADAM-12 deficient mice. ADAM-17/TACE is involved in the shedding of EGFR ligands (HB-EGF, TGFα) (58, 69) and the membrane-bound cytokine TNFαs (68). Adam-17−/−mice die at birth with a number of phenotypic changes including enlarged fetal hearts with increased cardiomyocyte cell size and ventricular cell proliferation (116), as well as abnormalities in lung development (117), hair and skin defects and failure of eyelid fusion (118). The hair and skin defects in these mice resembles TGFα-deficient mice (119, 120), and the cardiac defect is similar to that observed in mice lacking EGRF (121) or ErbB2, another tyrosine kinase receptor (122). ADAM-19/meltrin β is another ADAM essential for cardiovascular development and morphogenesis. Mice lacking this ADAM exhibit ventricular septal defects, immature valves leading to valvular stenosis, and abnormalities of cardiac vasculature. These mice die perinatally, possibly due to their cardiac defect (123, 124). ADAM-9/meltrin γ is highly expressed in developing mesenchyme, heart, and brain. However, mice lacking this ADAM develop normally and are viable and fertile with no major pathological phenotypes (113). Thus, ADAM-10, -17, and -19 are essential for embryonic cardiac development. Although no developmental cardiac phenotype has been reported in mice lacking ADAM-12 and ADAM-9, these genes may play a role in progression of heart disease. Based on studies to date, ADAMs play more important roles than MMPs in the cardiac development.

In conclusion, regardless of the cause of heart disease, alterations in ECM structure have an impact on cardiac architecture and function. Among the large number of metalloproteinases implicated in ECM remodelling, it is important to identify those that serve essential, non-redundant functions in this process. Understanding of these molecular factors is key to developing therapeutic strategies to prevent or treat heart disease.

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219


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