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Review

Interplay of matrix metalloproteinases, tissue inhibitors of metalloproteinases and their regulators in cardiac matrix remodeling

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Abstract

Myocardial fibrosis due to maladaptive extracellular matrix remodeling contributes to dysfunction of the failing heart. Further elucidation of the mechanism by which myocardial fibrosis and dilatation can be prevented or even reversed remains of great interest as a potential means to limit myocardial remodeling and dysfunction. Matrix metalloproteinases (MMPs) are the driving force behind extracellular matrix degradation during remodeling and are increased in the failing human heart. MMPs are regulated by a variety of growth factors, cytokines, and matrix fragments such as matrikines. In the present report, we discuss the regulation of MMPs, the role of MMPs in the development of cardiac fibrosis, and the modulation of MMP activity using gene transfer and knockout technologies. We also present recent findings from our laboratory on the regulation of the extracellular MMP inducer (EMMPRIN), MMPs, and transforming growth factor-β_1 in the failing human heart before and after left ventricular assist device support, as well as the possibility of preventing ventricular fibrosis using different anti-MMP strategies. Several studies suggest that such modulation of MMP activity can alter ventricular remodeling, myocardial dysfunction, and the progression of heart failure. It is therefore suggested that the interplay of MMPs and their regulators is important in the development of the heart failure phenotype, and myocardial fibrosis in heart failure may be modified by modulating MMP activity.

Keywords: Extracellular matrix; Gene therapy; Remodeling; Heart failure; Fibrosis

1. Introduction

Myocardial matrix remodeling has been proposed to participate in the development of ventricular dilatation and heart failure. Matrix metalloproteinases (MMPs), which are present in the myocardium and are capable of degrading all the matrix components of the heart, are the driving force behind myocardial matrix degradation during remodeling. Thus, an increase in MMP activity may result in fibrillar collagen degradation, extracellular matrix (ECM) remodeling, and progressive ventricular dilatation. The MMPs are regulated at both pre- and posttranscriptional levels, and can also be regulated by substrate interaction, and by endogenous physiological inhibitors [1,2]. Therefore, the interplay of MMPs, tissue inhibitors of metalloproteinases (TIMPs) and their regulators determines the progression of the fibrotic process in the heart. Modulation of this process may alter the final outcome of fibrosis and eventually, myocardial function. In the present report, we will discuss new insights into the expression and the regulation of MMPs and their regulators, and the effect of changes in their activity on cardiac structure and function.

2. Regulation of the expression and activity of MMPs

2.1. Regulation of MMP gene expression

MMP expression can be modified at the transcriptional level by a variety of physiologic signals including growth factors, cytokines, and matrikines. Each MMP gene has a unique promoter that contains various transcription factor

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binding sites. An activator protein (AP-1) site is present in the promoter of the MMP-1, -3 and -9 genes [3,4] and the MMP genes are highly inducible by phorbol myristate acetate (PMA) [4], possibly acting via the AP-1 site. A single AP-1 site is sufficient to drive transcription of the MMP genes in Hela cells, but not in fibroblasts [3,4]. This difference may be due to AP-1 complex formation by different Jun and Fos protein family members, which form heterogenous complexes that bind to AP-1 site with different affinities [5]. In fibroblasts, basal transcription of the MMP-1 gene is controlled by the proximal AP-1 sites, while transcriptional induction seen in response to PMA is mediated principally by several sequences in the proximal promoter [1]. Likewise, stimulation of MMP-9 gene promoter activity by Ras requires multiple transcription factor binding sites including polyoma enhancer activator-3 (PEA-3) and AP-1 [6].

Cytokines are important regulators of MMP gene expression. It has been shown that the expression of collagenase and c-jun is coregulated by tumor necrosis factor-α (TNFα) [7]. Both TNFα and interleukin-1β (IL-1β) induce a prolonged activation of c-jun gene expression, which may result in MMP gene activation through the AP-1 sites [8]. We have previously reported that MMP gelatinolytic activity in cultured neonatal cardiomyocytes and fibroblasts is increased by TNFα and IL-1β [9]. This change in MMP activity leads to a rapid decrease in ECM accumulation. IL-1β also increases the stability of MMP-1 transcripts resulting in higher levels of steady-state mRNA [10]. Thus both pre- and posttranscriptional mechanisms contribute to the increases in MMP gene expression in response to cytokines.

Transforming growth factor-β_1 (TGFβ_1) is also an important regulator of MMP gene expression. TGFβ_1 acts through the TGFβ_1 inhibitory element (TIE), a *cis*-acting element found in the promoter region of most MMP genes, with the exception of MMP-2 [11]. While TGFβ_1 suppresses overall proteolytic activity through reduced proteinase synthesis and by increased TIMP expression, it increases both MMP-2 and -9 expression in some cell types [12]. In addition, the effects of TGFβ_1 on the expression of MMPs are influenced by aging, and altered composition or modification of TIE binding factors in aging fibroblasts may underlie this effect [13].

In contrast to MMP-1, -3 and -9, MMP-2 is constitutively expressed at low levels by many cells [14]. For example, MMP-2 transcription is not readily induced by PMA or IL-1β, which may be explained by the absence of AP-1, PEA-3 and TIE binding sites in the MMP-2 promoter or the presence of a novel TATA box and a SP-1 consensus sequence [15]. However, high level transcription of MMP-2 is subject to the interplay of double (AP-2) and single-stranded (YB-1) DNA binding transcription factors with a discrete 40-base pair enhancer element (RE-1) located in the 5′-flanking region of the MMP-2 gene [16].

Extracellular matrix (ECM) metalloproteinase inducer (EMMPRIN), a member of the immunoglobulin superfamily, is a glycoprotein first identified on the outer surface of human tumor cells [17]. EMMPRIN stimulates human fibroblasts to produce MMP-1, -2, and -3, and upregulates MMP-1 mRNA expression in a concentration-dependent manner [17,18]. For MMP-1, this stimulating effect requires p38 activity [19]. Since previous studies have shown that MMPs are increased in the failing human heart [20,21], we hypothesized that EMMPRIN may be involved in the regulation of their expression. Relative to nonfailing hearts, the level of EMMPRIN protein was significantly elevated in failing human hearts (Fig. 1). This upregulation was independent of MMP-1, -2, -3 and -9 expression, as the increase in EMMPRIN did not correlate with changes in MMP-1 protein, MMP-2 activity, or MMP-9 protein and activity in the human heart (Fig. 1C,D) [22].

Because mechanical stretch has been shown to induce neutral transmembrane MMPs in cardiac fibroblasts [23], and MMP-2 and -9 are stimulated by transmural pressure [24], we hypothesized that EMMPRIN as well as other MMPs could be modulated in vivo by changes in hemodynamic load. To test this hypothesis, we used left ventricular tissue samples from failing human heart before and after left ventricular assist device (LVAD) support to study the regulation and relationship of EMMPRIN and MMPs. LVAD unloading for various durations led to a further increase in the expression of EMMPRIN. The fold induction in EMMPRIN levels correlated with the duration of LVAD support of the heart (Fig. 2A,B). However, MMP-2 activity did not change in the failing human heart compared to nonfailing controls, and in the LVAD supported heart compared to that before LVAD support. MMP-1 and -3 expression did not change, while MMP-9 decreased after LVAD support [22]. Thus, EMMPRIN expression alone does not appear to regulate MMP expression, while hemodynamic load appears to regulate MMP-9 in the human heart.

The expression of MMP genes, especially MMP-9, can also be altered by the substrates of MMPs, cell–cell and cell–ECM adhesion molecules, and agents that alter cell shape [25]. The laminin peptide, seryl-isoleucyl-lysyl-valyl-alanyl-valine (SIKVIV), induces MMP-9 in human monocytes, while intact laminin does not [26]. A fibronectin fragment that contains the central arginyl-glycyl-aspartate (RGD) cell binding region also induces MMP-9 expression [27]. Cells plated on a mixture of tenascin and fibronectin show upregulated expression of MMP-9 [28]. This response can be blocked by antibodies directed against β-integrins and by cytochalasin D, suggesting signal transduction through cell–cell contact and cytoskeletal components.

2.2. Storage of MMPs in the ECM

MMPs are synthesized and secreted as proenzymes

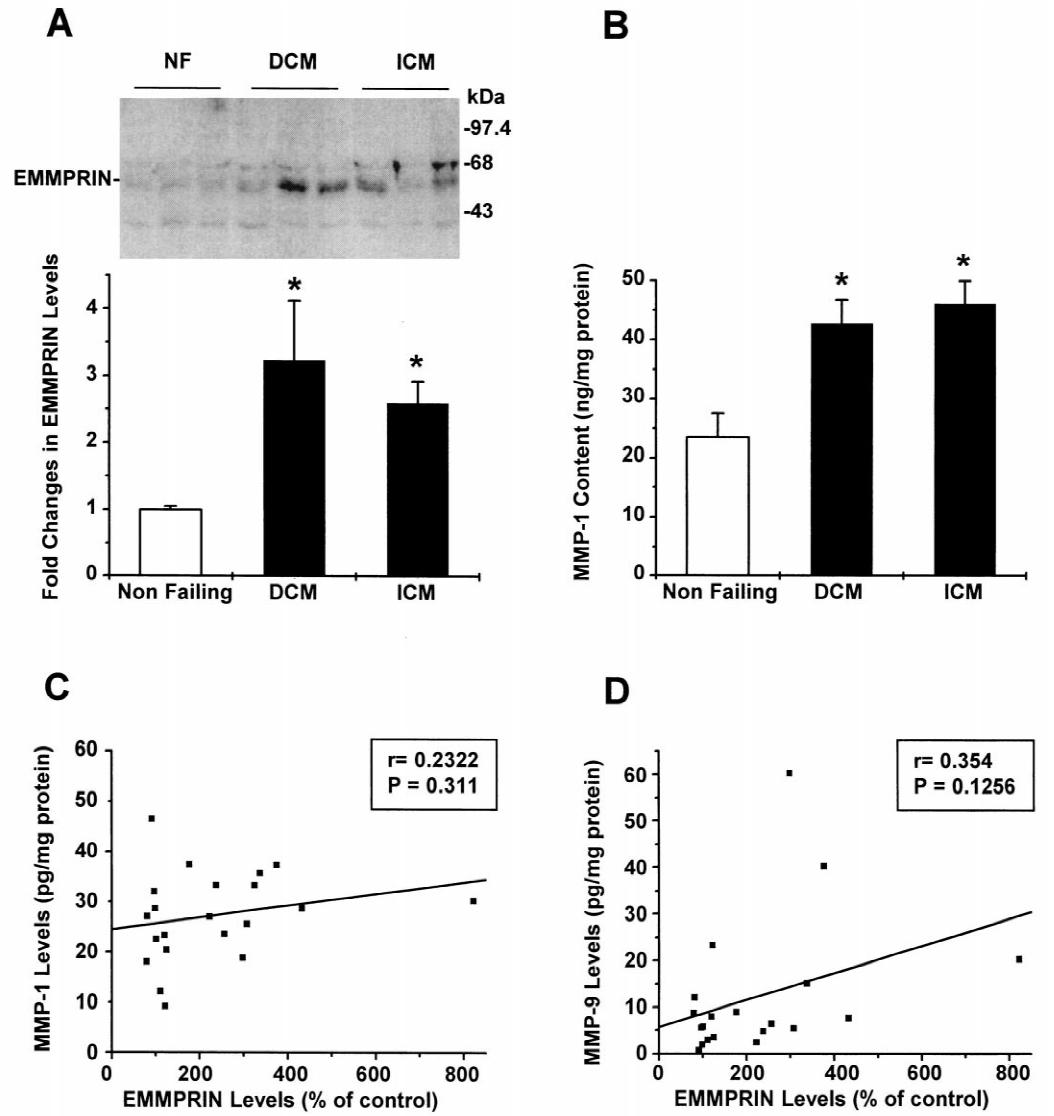

Fig. 1. The relationship of increased expression of EMMPRIN and MMPs. (A) Western blot analysis showed increased expression of EMMPRIN in both dilated cardiomyopathy (DCM, $n=7$) and ischemic cardiomyopathy (ICM, $n=8$) relative to non-failing heart ($n=8$). The protein expression of MMP-1 (B) and -9 (Ref. [21]) was also increased, but EMMPRIN protein did not correlate with either MMP-1 or -9 (C, and D). * $P<0.05$ compared to that of nonfailing heart.

(zymogens). After secretion the proMMPs bind to various ECM components, which may serve as a means of extracellular storage for rapid activation and mobilization upon stimulation. Interestingly, MMPs can display selective affinity with discrete components of the ECM. It has been demonstrated that MMP-2 is associated with elastin-containing structures, MMP-3 with basement membrane and occasionally with collagen fibers, and MMP-13 with proteoglycans, collagen and elastin [29]. proMMP-9 forms a high-affinity complex with α2(IV) chains on the cell surface to facilitate the surface/matrix association of proMMP-9 [30], whereas the binding of MMP-9 to α1(I) chains [31] may serve as a means of substrate targeted degradation.

2.3. Localized activation of MMPs

Although intracellular activation of proMMPs has been reported [32], the majority of proMMPs are secreted and stored in the ECM. The activation of proMMPs in the interstitium is mediated by both plasmin-independent and plasmin-dependent pathways [33]. Plasmin is a potent activator of most MMPs, promoting cleavage of the latent proenzyme to the active molecule [34]. Urokinase-like plasminogen activator (uPA) generated plasmin works with MMP-3 to activate MMP-9 [35]. proMMPs can be activated by removing the carboxyl terminus of the proenzyme. uPA associated with uPA receptor favors localized ECM degradation. It is suggested that the binding

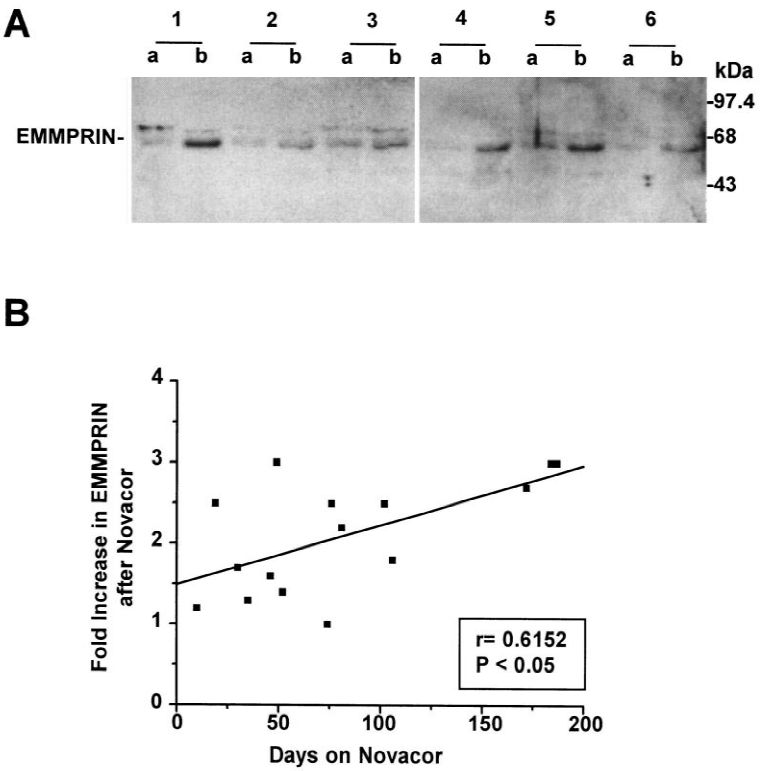

Fig. 2. The expression of EMMPRIN was further increased after LVAD support of the failing human heart. (A) Western blot analysis of EMMPRIN showed increased expression after LVAD support: a, before LVAD; b, after LVAD; (B) EMMPRIN increment correlated with the duration of LVAD support.

and activation of uPA on its receptor provide a mechanism for localized proteolytic activity at the cell. In a similar fashion, membrane type (MT)-MMPs activate MMP-2 on the cell surface with the MT1-MMP–TIMP-2 complex serving as a receptor for proMMP-2 [36], suggesting the presence of parallel transmembrane control systems for MMPs. The activated MT1-MMP and MMP-2 on the cell surface can serve as an activator of other MMPs such as MMP-13. This membrane-associated pathway is inducible by a variety of agents including collagen type I, concanavalin A or TGFβ. The concentration of TIMP-2 determines its role in localized activation of MMPs. At low concentrations, TIMP-2 serves as a receptor for proMMP-2, whereas at high concentrations, TIMP-2 neutralizes MT-MMP and prevents MMP-2 activation [36].

Local activation of MMPs is most readily facilitated by binding of collagens. The presence of soluble collagen stimulates collagenase and collagenolytic activity [37]. For example, MMP-13 expression and activity are induced in fibroblasts cultured on three-dimensional collagens [38], and monocytes cultured on type I collagen release more MMP-9 than do cells plated directly on plastic [39]. Type I collagen also induces dose-dependent posttranscriptional MMP-2 activation. Specific antibodies against the subunits of α2β1 integrins, the major collagen I receptor, partially inhibit MMP-2 activation [40].

2.4. Inhibition of MMPs

The MMPs are inhibited by TIMPs, synthetic compounds, as well as α2-macroglobulin [41]. TIMPs are secreted proteins with multiple functions. In addition to MMP inhibition, TIMP-2 inhibits cultured endothelial cell proliferation independent of protease inhibitory activity [42]. Growth-stimulatory activity has also been described for TIMP-1, -2, and -3 [43].

Because of the importance of ECM remodeling, there is a significant interest in utilizing MMP inhibition as a therapeutic strategy [44]. Since the activity of MMPs is increased, while TIMPs are decreased in the failing human heart [20,21,45], these interests have been directed at modulating the heart failure process through inhibition of activated MMPs. However, the TIMPs have not proven suitable for pharmacological applications due to their short half-life in vivo. Thus, synthetic inhibitors of MMPs have been developed and evaluated in animal models and initial results have been promising in improving cardiac pump function and blocking progression of heart failure [46]. A series of other low-molecular-weight MMP inhibitors, with varying efficacy and specificity of MMP inhibition, have been developed [47]. Batimastat (BB-94) was the first synthetic MMP inhibitor with a collagen-mimicking hydroxamate structure and was studied in humans. Chemically

modified tetracyclines were the first agents to obtain approval for clinical use in anti-MMP therapy of periodontal diseases [48]. However, their effects on cardiac remodeling remain undefined.

3. The effect of overexpression or deficiency of MMPs or TIMPs

3.1. Virus mediated gene transfer

Various recombinant viral vectors have been constructed that contain genes for MMPs or TIMPs and which achieve high levels of expression, usually through use of the cytomegalovirus major immediate early promoter. Several reports show that vascular smooth muscle cell functions can be regulated in vitro and in vivo by adenoviral MMP or TIMP gene transfer. It has been shown that adenovirus mediated expression of TIMP-1 inhibits smooth muscle cell migration and reduces neointimal hyperplasia in vascular balloon injury [49,50]. Furthermore, overexpression of TIMP-1, -2, or -3 mimics synthetic MMP inhibitors in inhibiting smooth muscle cell chemotaxis and invasion through reconstituted basement membrane [51]. Alternatively, syngeneic rat smooth muscle cells retrovirally transduced with TIMP-1 cDNA and seeded onto the luminal surface of the vessels resulted in local TIMP-1 overexpression, led to preserved elastin in the media, and prevented aneurysmal degeneration and rupture [52].

Adenoviral TIMP-2 gene transfer is also effective in reducing blood vessel neointimal thickening primarily by inhibiting MMP activity and smooth muscle cell migration in vitro and in vivo [53,54]. While the functional importance of TIMP-3 downregulation in the failing human heart [21] remains unknown, adenovirus mediated overexpression in model systems of heart failure may prove illuminating. TIMP-4 is the only known TIMP whose expression appears to be cardiac-specific [55]. TIMP-4 expression is upregulated in rat carotid arteries after balloon injury [56]; however, it is downregulated in the ischemic failing human heart [21]. The cardiovascular effect of TIMP-4 overexpression remains to be defined.

3.2. Transgenics

Transgenic technology has provided an opportunity to assess the effects of prolonged altered expression of MMPs and/or TIMPs in vivo [57]. Transgenic mice overexpressing MMP-7 in reproductive organs showed altered integrity of the ECM, cellular differentiation, and tissue-specific cellular destruction [58]. In addition, targeting of an autoactivating mutant of MMP-3 to mammary epithelia of transgenic mice resulted in progressive development of reactive stroma and increased collagen content [59], suggesting that enhanced MMP-3 activity is associated with increased collagen formation and fibrosis.

The potential importance of MMPs in the development of heart failure has recently been demonstrated by transgenic myocardial overexpression of MMP-1 in mice. Myocardial overexpression of MMP-1 produces left ventricular hypertrophy and hypercontractility in young mice and ventricular dilatation and failure in old mice ([60] and H.E. Kim, personal communication). Similarly, other transgenic mice with elevated myocardial MMP activity have demonstrated normal cardiac function in young mice and the development of ventricular dilatation and failure in older mice [61,79].

While TIMP proteins may not prove amenable to therapeutic purpose, transgenic expression of TIMPs may provide important insights into their role in matrix homeostasis and cardiac remodeling. However, to date such results have not been reported.

3.3. Gene knockouts

Gene ablation through homologous recombination (gene ''knockout'') has also been applied to the investigation of MMP and TIMP functions. Growth plates from MMP-9-null mice in culture show a delayed release of an angiogenic activator, establishing a role for this proteinase in controlling angiogenesis [25,62]. In addition, lack of MMP-9 gene partially protects against cardiac aneurysm rupture, while uPA inactivation completely protects against rupture after myocardial infarction [63]. Plasminogen-deficient mice show delayed posttransplant allograft arteriosclerosis, suggesting plasmin proteolysis is involved in accelerated arteriosclerosis by mediating elastin degradation, macrophage infiltration, media remodeling, medial smooth muscle cell migration, and formation of a neointima [64]. Gene knockout also provides important information about the activation of MMPs. For example, although activation of proMMP-9 was enhanced in the presence of plasmin(ogen), the activation of proMMP-2 or proMMP-9 is not affected by gene knockout of MMP-3, plasminogen activators or plasmin(ogen) [33,65], suggesting that in vivo activation of MMPs may occur via plasminogen-independent mechanisms.

No phenotype abnormality was originally reported in mice lacking the TIMP-1 gene [66]. Nevertheless, it was later demonstrated that mice deficient of TIMP-1 gene showed increased left ventricle mass and left ventricular end-diastolic volume [67]. In addition, female mice lacking TIMP-1 gene showed reduced levels of ovarian TIMP-2 and -3 mRNA, suggesting cross-regulation among TIMPs [66].

4. The role of MMPs and their regulators in the development of cardiac fibrosis

Cardiac fibrosis is defined not only as an increase in the concentration of matrix collagens in the interstitium, but

also changes in collagen type, organization and cross-links [68]. Reparative fibrosis is a result of a scarring process in which small and large areas of necrosis heal after direct insults such as infarction of the myocardium [68]. Reactive fibrosis may be a fibrogenic response of the myocardium to a variety of stimuli including chronic elevations in angiotensin II, mineralocorticoids, and immune complexes. In the ischemic failing human heart, multiple foci of reactive fibrosis account for more than two-thirds of fibrous tissue, whereas the infarct scar constitutes only one-third [69]. Collagen exists in several genetically distinct types. Type I and III are predominant in the myocardium with type I being predominant in the adult heart [70]. Increased collagen formation, with changes in the ratio of type I to type III, may occur in response to a variety of growth signals. Various changes in the composition of collagen types and cross-links have been reported during the development of cardiac fibrosis in different animal models as well as in patients with heart failure [71–74]. However, the mechanisms involved in the differential regulation of the two collagen types during cardiac fibrosis appear to be complex and diverse [75]. These changes may lead to alterations in the mechanical properties of the tissue. For example, myocardial compliance ($\Delta V/\Delta P$) is directly affected by the concentration as well as the ratio of different types of collagens in the heart [74,76]. Elevations in type I collagen increase myocardial stiffness, while increases in type III collagen may facilitate myocardial compliance. However, myocardial stiffness in hypertension is proposed to be the consequence of an enhanced myocardial collagen cross-linking rather than an increase in total or type I collagen concentrations [77]. The normal collagens in the failing heart are degraded by increased MMPs and are replaced by fibrous interstitial deposits of poorly cross-linked collagens [78], which may lead to dilatation of the ventricles.

4.1. MMPs in myocardial fibrosis

MMPs not only play a role in the degradation of matrix components, but also modulate collagen synthesis. The end result is often increased MMPs accompanied with increased fibrosis such as seen in the failing heart, and decreased MMP activity accompanied with reduced fibrosis [22,63,79]. MMPs may participate in the fibrosis and remodeling process through direct digestion of matrix components, and regulation of the formation of matrikines: such as glycyl–histidyl–lysine [80] and release of biologically active factors from the ECM (including $TGF\beta_1$, insulin-like growth factor, and fibroblast growth factor [81]).

The direct digestion of matrix components is an essential part of matrix remodeling. MMPs have preferred substrate specificities (Table 1). MMP-1 initiates the digestion of collagens by hydrolyzing the peptide bond following a Gly residue located at a distance of three-fourths of the collagen molecule length from the amino terminus [82]. The resulting three-fourths and one-fourth fragments are completely degraded by MMP-2 and -9 in addition to MMP-1 and -3. In coronary artery ligation induced myocardial infarction, collagen degradation exceeds synthesis during the early phase of repair at the infarct site. Increases in collagenase and gelatinolytic activities appear at the infarct site on day 2 postligation, peak by day 7, and decline thereafter. An increase in collagenase mRNA expression which appears at day 7 may serve to replace the consumed latent MMP pool in the ventricles [83].

Digestion of the ECM releases molecules with potent effects on matrix synthesis, such as matrix bound growth factors as well as matrikines. Matrikines are fragmented matrix peptides that have biological activities in regulating connective tissue cell activity [80,84]. MMPs play an active role in the formation of some of the matrikines. The tripeptide glycyl–histidyl–lysine derived from several ECM proteins [including collagen $\alpha2(I)$, $\alpha2(V)$, and $\alpha2(IX)$ chains, osteonectin, thrombospondin-1 and fibrin α chain] during their partial degradation stimulates new connective tissue formation [80]. Peptides derived from elastin [85], laminin and fibronectin [86], and osteonectin [87] also participate in the modulation of cell activities, MMP expression, and growth factor signaling. The increase in mRNA of both type I and type III procollagens after infarction [88] may result at least partially from matrikines. Therefore, MMP facilitated formation of matrikines may play an active role in the regulation of fibrogenic process.

In addition to matrikines, MMPs may release additional biologically active factors that are associated with components of the ECM or cell membrane. A variety of growth factors have been found associated with particular components of the ECM: platelet derived growth factor (PDGF) with osteonectin; active $TGF\beta_1$ with collagen IV and fibronectin; and insulin-like growth factor with multiple IGF binding proteins.

The role of MMPs in myocardial fibrosis can be best exemplified by the findings of Heymans et al. [63]. Either uPA inactivation or adenoviral mediated TIMP-1 or plasminogen activator inhibitor (PAI) overexpression reduces collagen deposition in the infarcted heart. By contrast, individual inactivation of MMP-3, -9 or -12 genes has no effect. These findings suggest a redundant and cooperative role among MMPs in myocardial fibrosis.

4.2. Growth factors and cytokines in myocardial fibrosis

Myofibroblasts are specialized fibroblasts that express receptors for $TGF\beta_1$, angiotensin II, endothelin and proinflammatory cytokines, suggesting the ability to respond to these regulatory factors [89]. Indeed, angiotensin II generated de novo within the infarcted heart has autocrine and paracrine properties that influence the turnover of connec-

Table 1
Major matrix metalloproteinases and their properties

MMP	Traditional name	Major substrates	Activator	Inhibitors (beside EDTA)	Inducers[a]	Major cell or tissue distribution
MMP-1	Interstitial collagenase	Collagen I, II, III, X gelatin	Plasmin MMP-3, -7, -10	TIMP-1, -2, -3, -4 tetracycline	TNFα, IL-1β PDGF, phorbol	Widely including heart
MMP-2	Gelatinase A	Gelatin, laminin collagen I, IV, V, VII fibronectin, elastin	MT-MMP	TIMP-1, -2, -3, -4	TGFβ	Ubiquitous
MMP-3	Stromelysin	Proteoglycans fibronectin, gelatin collagens III, IV, V, IX		TIMP-1, -2, -3, -4	TNFα, IL-1β, EGF phorbol	Heart, lung, liver
MMP-7	Matrilysin	Gelatin, fibronectin proteoglycan		TIMP-1, -2, -4	LPS	Postpartum uterine
MMP-8	Neutrophil collagenase	Collagens I, II, III gelatin	MMP-3, -7, -10	TIMP-1, -2	TNFα, IL-1β	Neutrophils, postpartum uterine
MMP-9	Gelatinase B	Gelatin, proteoglycans collagens IV, V, VII fibronectin, elastin	Plasmin MMP-2	TIMP-1, -2, -3, -4 tetracycline	TGFβ, TNFα, IL-1β LPS, phorbol, EGF ischemia cell-matrix contact	WBC, osteoclast, trophoblasts
MMP-10	Stromelysin-2	Gelatin		TIMP-1, -2		Heart, lung, liver, intestine
MMP-11	Stromelysin-3	Gelatin		TIMP-1, -2		
MMP-12	Metalloelastase	Elastin		TIMP-1, -2		Placenta, Mϕ, stromal cells
MMP-13	Rodent collagenase	Collagen I, II, III	MT-MMP		LIF, TNFα, IL-1β phorbol	Widely including heart postpartum uterine
MMP-14	MT1-MMP proMMP-2	Collagen, aggrecan		TIMP-2, -3	TNFα, IL-1β, EGF phorbol, ConA	Lung, kidney, spleen, placenta
MMP-15	MT2-MMP	Collagen, aggrecan			Stretch	Heart, lung, liver, colon, kidney
MMP-16	MT3-MMP					Brain, lung, placenta
MMP-17	MT4-MMP					Heart, brain, colon, WBC, ovary
MMP-18	Collagenase-4	Collagen				
N/A	MT5-MMP	proMMP-2				Brain

[a] TGFβ=transforming growth factor β; TNFα=tumor necrosis factor α; IL-1β=interleukin-1β; LPS=lipopolysaccharide; EGF=epithelial growth factor; PDGF=platelet derived growth factor; LIF=leukemia inhibitory factor; WBC=white blood cell; ConA=concanavalin A; Mϕ=macrophage.

tive tissue [89]. The stimulating effects of TGFβ on collagen gene transcription have been documented in cardiac fibroblasts [90]. In infarcted rat heart, locally generated angiotensin II is correlated to $TGF\beta_1$ expression and synthesis [91]. It is proposed that early induction of $TGF\beta_1$ via the angiotensin II type 1 receptor plays a major role in the development of cardiac fibrosis [92]. $TGF\beta_1$ treatment of cardiac fibroblasts increases the abundance of proα2(I) and proα1(III) mRNA and type I and type III collagens [90]. Similarly, recombinant adenovirus mediated overexpression of $TGF\beta_1$ results in elevated levels of type III collagen gene expression in vascular smooth muscle cells and fibroblasts [93]. Consistent with in vitro and animal studies, we have found that $TGF\beta_1$ levels correlate with the deposition of collagens in the human heart (Fig. 3), suggesting a role of $TGF\beta_1$ in the regulation of human myocardial fibrosis.

Proinflammatory cytokines such as TNFα and IL-1β can both enhance and inhibit collagen production in lung fibroblasts [94]. In cultured neonatal rat cardiomyocytes, TNFα and IL-1β stimulate the activity of MMPs and accelerate the breakdown of matrix proteins within 48 h of exposure [9]. However, in the intact heart different long term effects may occur due to (1) the collagen synthesis-promoting effects of matrikines generated by digestion of ECM as well as (2) the stimulating effect of those cytokines on the production of $TGF\beta_1$. This is demonstrated by studies in transgenic mice overexpressing TNFα (TNF1.6) that develop myocardial fibrosis and failure [61]. The mouse heart had significantly increased activity of MMPs [79] and expression of TGFβ, which may coordinately participate in the fibrogenic process. As a result, the

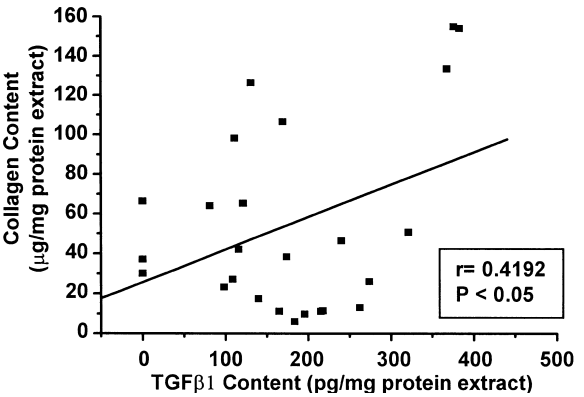

Fig. 3. Correlation of the levels of $TGF\beta_1$ with collagen deposition in the human myocardium. Myocardial $TGF\beta_1$ content was measured by enzyme-linked immunosorbent assay. Myocardial collagen deposition was measured by hydroxyproline quantification.

net accumulation of collagens may increase. Indeed the myocardium of TNF1.6 mice shows increased collagen matrix [79]. Immunostaining of type I and III collagens also showed that the proportion of type III collagen was markedly increased in TNF1.6 transgenic mice [79].

5. Preventability or reversibility of fibrosis in the failing heart by modulating MMP activity

Regardless of etiology, heart failure often culminates in a presentation of cardiac fibrosis, dilatation, and loss of contractility. Currently there is much interest in the mechanism and preventability of cardiac fibrosis and dilatation. Modulation of the renin–angiotensin system by angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers alters the progression of myocardial fibrosis [95,96]. It has also been shown that inhibition of the renin–angiotensin system reverses cardiac fibrosis in deoxycorticosterone acetate (DOCA)-salt rats and returns some indices of myocardial function to normal [97]. Myocardial fibrosis is a major pathological finding that may be involved in both systolic and diastolic dysfunction of the failing heart. In fibrotic myocardium, collagen chains are fractured and replaced by poorly structured bands and sheets of collagen, or more soluble matrix components, which leads to ventricular dilatation [98]. This constant remodeling of the ECM is regulated by the activity of MMPs [89], which in turn are regulated by growth factors and cytokines. For example, the expression of TNFα and IL-1β is increased in patients with heart failure [99], and experimental studies have demonstrated that the gelatinolytic activity of MMPs is increased in cardiac as well as other cells after stimulation by these cytokines [9,100]. Therefore, modulation of the myocardial remodeling process and ultimately myocardial function could be achieved by changing the activity of MMPs either by direct inhibition or by anti-cytokine treatment.

Indeed, anti-TNFα treatment with ENBREL (Etanercept, p75 TNF receptor Fc fusion protein) has been reported to regress left ventricular remodeling and dilatation in humans with congestive heart failure [101]. We hypothesized that changes in the activity of MMPs may participate in this regression. To test this hypothesis, we used our TNF1.6 heart failure model to study the activity of MMPs, extent of collagen deposition, and cardiac function after inoculation with an adenovirus allowing systemic overexpression of a similar human TNF receptor type I-murine IgG fusion protein (AdTNFRI). We observed that the activity of MMP-2 and -9 in the TNF1.6 mouse heart was significantly increased and was effectively reduced by AdTNFRI. Along with suppression of the activity of MMPs in the heart, the total collagen deposition was also reduced after 6 weeks. We propose that the activity of MMPs and collagen content are tightly coregulated, and suggest that accumulated collagen substrate or its derivatives may trigger the activation of MMPs. Furthermore, the changes in MMP activity and collagen content are associated with myocardial diastolic function. In the TNF1.6 mice, increased MMP activity and collagen content are associated with decreased transmittal Doppler echocardiography E wave and A wave ratio (E/A ratio), whereas decreased MMP activity and collagen content after AdTNFRI treatment were associated with normalized E/A ratio [79].

A more direct inhibition of MMPs was recently reported [46] in which the MMP inhibitor PD166 793 (Parke-Davis) was used in a porcine model of heart failure induced through rapid ventricular pacing. The inhibitor treatment increased the endocardial shortening and left ventricular myocardial stiffness, reduced end-diastolic dimension and left ventricular wall stress and myocyte length. Administration of a broad-spectrum MMP inhibitor (CP-471 474) attenuates early left ventricular dilatation after experimental myocardial infarction in mice [102]. Furthermore, the effects of MMP inhibition on end-systolic area and end-diastolic area are most prominent in animals that had greater initial left ventricular dilatation.

6. Conclusion and perspectives

The myocardial ECM is under constant remodeling by MMPs, which are in turn regulated by various factors. Although significant advancement has been made in the understanding of the roles of MMPs, TIMPs and their regulators in the cardiovascular system, there is still much to be learned about the interaction of MMPs and their regulators in the development of myocardial fibrosis and the heart failure phenotype. From the current evidence we have, it is reasonable to believe that modulation of MMPs in the failing heart directly or through factors that affect MMP activity will alter the ECM remodeling process, which may eventually alter the progression of heart failure. Thus, the MMPs and TIMPs may provide an important therapeutic target for the discovery of new drugs for treating heart failure.

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