Chapter 2

Heart Remodelation: Role of MMPs

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http://dx.doi.org/10.5772/intechopen.71662

Abstract

Myocardium is comprised of a number of cell types. Although most plentiful by volume, cardiac myocytes are greatly outnumbered by nonmyocyte cells, the latter constituting approximately 70% of all myocardial cells, of which approximately 90% are cardiac fibroblasts (CFBs). To maintain the integrity of the cardiac extracellular matrix (ECM) is one of the primary functions of cardiac fibroblasts. ECM represents a network structure that provides the structural and functional integrity to the heart. Besides that, it also contains a high number of cytokines and growth factors with effects on cardiac function and cardiac cells. Cardiac ECM also mediates the mechanical connection between the cardiomyocytes, CFBs, and blood. In addition to producing ECM proteins, CFBs also produce ECM-regulatory proteins – matrix metalloproteinases (MMPs), which can degrade ECM proteins – and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs). To date, 26 MMPs have been cloned and characterized in vertebrates. From these, MMP1, MMP3, MMP8, MMP13, MMP2, MMP9, MMP12, MMP28, and the membrane-type MMPs (MT1-MMP/MMP14) have been identified to be involved in the myocardial remodeling. The role of higher MMPs in the cardiovascular system is less well explored.

Keywords: MMPs, heart remodeling, heart failure, acute coronary syndrome, atherosclerosis

1. Introduction

The heart is a muscular pump composed of cardiac myocytes and interstitial components. Although most plentiful by volume, cardiac myocytes are greatly outnumbered by nonmyocyte cells, the latter constituting approximately 70% of all myocardial cells, of which approximately 90% are cardiac fibroblasts (CFBs) [1]. Whereas cardiac myocytes and the coronary vasculature are central to the contractile function and viability of the myocardium, so too is the extracellular matrix, or cardiac interstitium serve as supporting structure. Collagen fibers, the major structural proteins of the interstitium, serve several functions showed in **Table 1** [2].

Scaffold supporting muscle cells and blood vessels
Lateral connections between cells and muscle bundles to govern architecture
Acting as a signal transducer for cell-cell communication modulating cell motility, survival, and cell proliferation
Coordinating the delivery of force, generated by myocytes, to the ventricular chamber
Determinants of diastolic and systolic myocardial stiffness
Serving to resist myocardial deformation and maintaining shape and wall thickness

Table 1. Functions of the collagen matrix in the heart.

The ECM is crucial to maintain appropriate cardiac integrity and pump function. Conversely, disruption of ECM homeostasis is a central factor for cardiac dysfunction, pathologic remodeling, and fibrosis following cardiac injury.

Most myocardial collagen fibers consist of collagen types I and III, which (depending on species) account for approximately 80% and 10% of collagen in the healthy heart, respectively. The nonstructural compartment of the ECM houses a variety of proteins, which are vital for ECM plasticity, and can be divided into three major groups: glycoproteins, proteoglycans, and glycosaminoglycans [3].

In addition to a fibrillar collagen network, a basement membrane, proteoglycans, and glycosaminoglycans, the myocardial ECM contains a large reservoir of bioactive molecules. For example, the concentration of angiotensin II (ANG II) and endothelin (ET)-1 is over 100-fold higher within the myocardial interstitium than in plasma. ECM also acts as a reservoir for growth factors (such as transforming growth factor-β), which are stored within the myocardial interstitium in a latent form and directly influence myocardial ECM synthesis and degradation. Moreover, mechanical stimuli such as stress or strain are transduced through the myocardial ECM to the cardiac myocyte, which in turn would directly affect myocyte growth and ECM remodelation [4, 5].

2. Normal heart interstitium

2.1. Collagens

As mentioned above, collagen is the predominant structural component of the ECM. It has been classified into three components:

1. epimysium – the collagenous matrix that lies below the endothelium of the epicardium and endocardium and surrounding the entire muscle
2. endomysium – endomysial collagen fibers consist of fibrils that connect adjoining myocytes to one another and to their neighboring capillaries
3. perimysium – surrounding and interconnecting groups of myocytes. It consists of tendon-like extensions of the epimysium that arborize into a weave to aggregate myocytes into myofibers [6].

Collagen synthesis and degradation in the healthy heart is an ongoing balanced process. The major types of collagen present in the myocardium of the left ventricle are I and III, with type I predominating. Type I collagen is the most abundant collagen of the human body, which forms large, eosinophilic fibers known as collagen fibers. The COL1A1 gene produces the pro-alpha1 (I) chain, and pro-alpha2 (I) chain is produced by the COL1A2 gene. Two chains of pro-alpha1 (I) combine together with one pro-alpha2 (I) chain to make a molecule of type I procollagen. These molecules must be processed by enzymes outside the cell. After that, they arrange themselves into long, thin fibrils that cross-link to one another in the spaces around cells, which leads to the formation of very strong fibers of the mature type I collagen. In humans, collagen alpha-1 (III) chain is encoded by the COL3A1 gene located on chromosome 2. Collagen alpha-1 (III) chain is a precursor for collagen III that is found in extensible connective tissues [7, 8].

Myocytes are surrounded by a basement membrane (BM). The principal structural component of the basement membrane is collagen type IV, while collagens I and III are arranged in sequential layers of organization of the BM. However, collagen does not only fulfill the architectural function. It is also involved in intracellular signal transduction. Via a β1 integrin-dependent mechanism, collagen can inhibit apoptosis and so promotes cell survival in vitro. Collagen is also implicated in the induction of proliferation via FAK activation and downstream signaling pathways (Src, MEK, PI3-kinase, and p38 MAPK). In addition, collagen participates in cell spreading through p130Cas phosphorylation via FAK-dependent and FAK-independent integrin receptor pathways [9, 10]. Finally, collagen plays a key role in cell migration through the activation of FAK and PI3-K, leading to elevated Rac1 activity as a downstream consequence in activated cell migration [11].

2.2. Cardiac fibroblasts

Cardiac myocytes occupy approximately 75% of normal myocardial tissue volume, but they account for only 30–40% of cell numbers. Cardiac fibroblasts (CFBs) have the highest cell population in the myocardium, accounting for about two-thirds of the cells.

Fibroblasts (FBs) are present in every tissue in the body [5]. They are of mesenchymal origin and are morphologically flat and spindle-shaped, with multiple processes. CFBs in the myocardium lack a basement membrane, which distinguishes them from all other cells. In the past, FBs have been considered for a homogeneous cell population. Over time, though, it has become clear that FBs from various tissues have different properties and functions. Fibroblasts are found throughout the cardiac tissue, surrounding myocytes and bridging "spaces" between myocardial tissue layers, so that, in essence, every cardiomyocyte is closely related to a fibroblast in normal cardiac tissue. The primary function of FBs is to produce structural proteins that form the extracellular matrix. Under physiological circumstances, this is a constructive process. On the other site, hyperactivity of cardiac fibroblasts can result in excess production and deposition of ECM proteins that can lead to fibrosis. Fibrosis of the myocardium affects the cardiac structure and function in the negative way. Fibroblasts also produce a number of cytokines, peptides, and enzymes including matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs) that directly affect the ECM turnover and homeostasis. In turn, as a feedback, function of fibroblasts can also be regulated by MMPs and TIMPs [12, 13].

CFBs can differentiate into myofibroblasts (myoFBs), more mobile and contractile elements with a greater synthetic potential to produce ECM proteins. Incentives for this differentiation are various stimuli, most commonly myocardial injury. MyoFBs were identified by Gabbiani in 1971 [14]. They only appear after cardiac injury and are not found in healthy myocardium. Cardiac myoFBs, similar to CFBs, are nonexcitable cells, which express a number of smooth muscle cell markers, such as alpha smooth muscle actin (αSMA), smooth muscle myosin heavy chain, paxillin, vinculin, and tensin, that are not typically expressed in quiescent CFBs [15].

2.3. Matrix metalloproteinases in the normal heart

MMPs are a family of more than 25 species of zinc-dependent proteases that are synthesized as inactive zymogens (pro-MMPs). They are essential for normal tissue remodeling in processes such as bone growth, wound healing, and reproduction. Moreover, increased induction and elaboration of MMPs have been identified to hold biological significance in a number of pathological conditions that include cancer, inflammatory disease, and cardiovascular disease. MMPs are the predominant proteases responsible for degradation of the ECM proteins. To date, 26 MMPs have been cloned and characterized in vertebrates. From these, MMP1, MMP3, MMP8, MMP13, MMP2, MMP9, MMP12, MMP28, and the membrane-type MMPs (MT1-MMP/MMP14) have been identified to be involved in the myocardial remodeling. The role of higher MMPs in the cardiovascular system is less well explored [16].

The interstitial collagenase (MMP-1), neutrophil collagenase (MMP-8), and collagenase-3 (MMP-13) possess high substrate specificity for fibrillar collagens, as well as other ECM proteins such as aggrecan, perlican, versican, and proteoglycans. The important substrates for MMP-1 and MMP-13 within the myocardium include the fibrillar collagens such as collagen type I and III [17].

The gelatinases (MMP-2 and MMP-9) demonstrate substrate affinity for denatured fibrillar collagen, basement membrane proteins such as collagen type IV, fibronectin, and laminin. MMP-2 and MMP-9 also exhibit proteolytic activity against elastin and proteoglycans. Past studies have demonstrated that MMP-9 is synthesized by myocytes, fibroblasts, and smooth muscle cells. Moreover, neutrophils have also been reported to be a potential source of MMP-9 [18].

Stromelysin (MMP-3) degrades all basement membrane proteins, elastin, and proteoglycans.

MMP-7 lacks the hemopexin domain, which seems to be critical in substrate recognition. Therefore, MMP-7 has a wide substrate portfolio and possesses proteolytic activity against the fibrillar collagens I and III, basement membrane proteins (collagen IV and fibronectin), and proteoglycans [19].

MMP-12 (macrophage elastase) has broad substrate specificity for extracellular components and is shown to be a key player in tissue remodeling associated with many pathological conditions such as chronic inflammation and fibrosis [20].

During ECM degradation (in physiological ECM turnover or pathological ECM remodeling), collagen fibers are degraded into smaller peptides. After that, telopeptides in the N-terminals or C-terminals of collagen molecules are cleaved. The propeptide from the carboxy-terminal

or the amino-terminal propeptides of collagen type I (PICP, PINP) and both terminals of collagen type III (PIIICP, PIIINP) that uprise during biosynthesis of these collagens can be considered as biomarkers of collagen synthesis, as they are released in a stoichiometric manner. Similarly, the C-terminal or N-terminal telopeptides of collagen type I (CITP, NITP) and type III (CIIITP, NIIITP), produced by degradation of these collagens, are considered biomarkers of collagen degradation [21].

3. MMPs in heart disease

Matrix metalloproteinases and their inhibitors have a fundamental role in the remodeling of the ECM in both normal and pathological conditions. In addition, MMPs have an important role in cardiovascular diseases, including acute and chronic heart failure (CHF), acute myocardial infarction, atherosclerosis, and cardiomyopathies.

3.1. MMPs in acute heart failure

Although the relationship between MMPs and chronic heart failure (CHF) has been well investigated, we have poor information about changes in the serum levels of MMPs in patients with acute heart failure (AHF). In patients with AHF, production of MMPs is affected by several mechanisms including changes in hemodynamic conditions and neurohormonal and inflammatory factors. Biolo et al. reported that some markers of extracellular matrix turnover (MMP-2, TIMP-1, and procollagen type III N-terminal peptide) were elevated in patients with AHF syndrome. The increase in ECM turnover may be associated with an acceleration of pathological remodeling. The decrease in the concentrations of MMP-2 during the acute phase of heart failure may represent the deceleration of myocardial remodeling and maybe a better prognosis of AHF [22]. In the study of Shirakabe et al., the serum levels of MMP-2 were measured on 83 AHF patients before starting treatment (day 1), on day 3 and day 7 after admission to the hospital, and before discharge. They found rapid and significant decrease in the MMP-2 concentrations on day 3 compared to day 1, whereas the MMP-2 levels were not significantly different on day 7 and at predischarge. Authors also evaluated the relationship between ΔMMP-2 (defined as the changes in MMP-2 concentrations from day 1 to day 3) and HF events including cardiac death, readmission to hospital for HF, and controllable HF. The levels of MMP-2 were significantly ($p = 0.004$) more decreased in the event-free group compared to the group with events mentioned above. The results of multivariate logistic regression model for predicting HF events found that the specific factor for HF events was ΔMMP-2. Rapid decrease in MMP-2 concentration after acute heart failure event thus may be important for better clinical outcome in patients with AHF [23].

Hadipurnomo et al. examined samples of 122 consecutive patients with acute coronary syndrome (ACS) treated in ICCU of which 47 showed the signs of AHF. The level of MMP-9 was examined a time at admission in ICCU, before thrombolysis was done. The acute heart failure accompanying ACS had Killip II-IV scores. The level of MMP-9 in ACS with AHF was significantly higher than in ACS without AHF, with p value <0.001 [24]. Similar results were

presented in a study of Jong et al. who investigated the serum concentrations and activities of MMP-9 in patients with heart failure developed after AMI. Twenty-eight patients post-AMI and without heart failure (Killip I, cardiopulmonary compensated) and twenty-seven post-AMI patients who developed heart failure (Killip II-III, decompensated) were selected to evaluate the serum levels and activities of MMP-9. It was observed that both serum levels and activities of MMP-9 significantly increased ($P < 0.01$) in decompensated group compared to compensated group. The highly elevated serum MMP-9 concentration of decompensated patients was not related to inflammatory or localized infarct area of myocardium. Authors suggest that the increase of MMP-9 levels and activity may be used as a new marker to diagnose the development of heart failure in patients post-MI [25]. Levosimendan is calcium sensitizer, cardiotonic agent that binds to troponin C with high affinity. This pharmaceutical agent promotes cardiac contractility without increasing myocardial oxygen demand. Tziakas, et al. demonstrated that levosimendan significantly reduced MMP-2 levels in patients with acute decompensation of chronic heart failure [26].

3.2. MMPs in the chronic heart failure

Essential points in the development and progression of HF include changes in the structure, composition, and geometry of the left ventricular (LV) myocardium, which has been generically termed LV remodeling. Left ventricular remodeling that precedes and occurs along the development of HF is strongly associated with adverse clinical outcomes in HF patients with systolic dysfunction. Changes in the overall structure and function of extracellular matrix directly contribute to the adverse LV remodeling. There are fairly distinct patterns of LV remodeling that occur and are dependent on the initial pathophysiological stimulus but, once instigated, LV remodeling is an important predictor for the development and progression of HF. The activity of MMPs has been shown to be increased in the progression of heart failure. The level of MMPs and their induction and activation systems are increased in pathological specimens of human heart failure.

As mentioned above, sources of MMPs in the heart are fibroblasts, myocytes, endothelial cells, and inflammatory cells, e.g. monocytes that infiltrate the myocardium in several circumstances. In heart failure, plasma levels of tumor necrosis factor-α (TNF-α) are increased. Monocytes stimulated by TNF-α are capable to produce MMP-9. It is supposed that monocytes and other blood elements may serve as carriers of MMP-9, which is synthesized before they infiltrate the myocardium. Observation that in reperfusion of myocardium after acute infarction, infiltrating neutrophils are the predominant source of MMP-9 and activating enzymes supports this hypothesis.

Cyclic strain has been shown to induce a number of MMPs, such as the gelatinase MMP-2. In myocardial biopsies performed in patients with LV pressure overload secondary to aortic stenosis, increased MMP-2 expression and activity were identified. In patients with LV hypertrophy and HF with a history of hypertension, plasma levels of MMP-2 were significantly increased compared with age-matched control subjects. For example, in the study of Spinale et al., left ventricular myocardial MMP activity (measured by zymography) increased by >2-fold in nonischemic dilated cardiomyopathy (DCM) and ischemic DCM when compared

with MMP activity in normal hearts. Abundant concentration of MMP-9 was observed in both forms of DCM. MMP-2 and MMP-3 activities were increased with nonischemic DCM. On the other site, MMP-1 levels were decreased in both forms of DCM [27].

In patients with CHF, elevated serum levels of MMP-1 and TIMP-1 have been observed. This finding indicates a predominance of collagenolytic activity, which results in an increase of serum concentrations of CITP (type I collagen carboxy-terminal telopeptide), a marker of collagen degradation. A report from López et al. shows an increase of the MMP-1/TIMP-1 ratio (both in tissue and serum samples) in hypertensive systolic heart failure compared with hypertensive diastolic heart failure [28]. On the other hand, some other studies analyzed human myocardium from explanted hearts from patients undergoing heart transplantation. They have found decreased MMP-1 expression and notable increase in TIMP-1 concentration [29]. Finally, study of George et al. found that MMP-2 but not TIMP-1 is an independent predictor of mortality in patients with CHF [30]. In the study of Jordán et al., patients with CHF had lower levels of MMP-1 and higher levels of TIMP-1 and TIMP-1/MMP-1 ratio than controls. TIMP-1 levels and the TIMP-1/MMP-1 ratio correlated negatively with peak VO2. They also described higher baseline peak VE/VCO2, TIMP-1, TIMP-1/MMP-1 ratio values, and lower MMP-1 levels in patients who suffered endpoints (total mortality, readmissions for heart failure, and cardiac transplantation). On multivariate analysis, VE/VCO2, MMP-1 levels, and age were the only variables independently related to prognosis of these patients [31]. Similarly, detectable plasma levels of MMP-13 were reduced in patients with LV hypertrophy and HF [32]. Interestingly, transgenic expression of human MMP-1 in mice (this MMP type is absent in rodents) and induction of LV pressure overload resulted in a relative reduction in myocardial fibrillar collagen content and improved indices of LV function [33]. These findings suggest that the loss of normal constitutive levels of certain MMP types, or failure of an induction of certain MMP types with LV pressure overload, may facilitate abnormal ECM accumulation and adverse myocardial remodeling.

Study of Morishita et al. demonstrated that in patients with heart failure with preserved ejection fraction (HFpEF), levels of BNP and the MMP-9/TIMP-1 ratio were lower compared to those with heart failure with reduced ejection fraction (HFrEF). An imbalance in the MMP/TIMP ratio and a robust increase in BNP levels reflect advanced ventricular remodeling, dilatation, and wall stretching. MMP and TIMP levels were similar in HFrEF and HFpEF patients and may represent ongoing myocardial injury and extracellular matrix remodeling before an increase in BNP and a decreased ejection fraction are seen. HFpEF is characterized by matrix apposition and myocardial stiffening [34]. Similarly, Martos et al. studied hypertensive patients divided into groups according to the presence of HFpEF and phase of diastolic function. Serum carboxy-terminal telopeptide of procollagen type I, carboxy-terminal telopeptide of procollagen type I, amino-terminal propeptide of procollagen type III, MMP-2, and MMP-9 levels were greater in patients with HFpEF than in those without. When controlled for age and gender, levels of serum carboxy-terminal telopeptide of procollagen type I, tissue inhibitor of MMP-1, amino-terminal propeptide of procollagen type III, concentrations of PICP (carboxy-terminal telopeptide of procollagen type I), and MMP-2 were increased in more severe phases of diastolic dysfunction. Within phases of diastolic dysfunction, markers of collagen production (such as serum carboxy-terminal telopeptide of procollagen type I,

amino-terminal propeptide of procollagen type III) and MMP-2 and MMP-9 were elevated in those with HFpEF compared to those without signs of heart failure. Thus, a matrix and fibrosis markers such as MMPs may also be an important prognostic markers in HFpEF [35].

On the other hand, many clinical studies have quantified various circulating levels of MMPs and TIMPs in patients with HFrEF. MMP-8 levels were decreased in HF patients compared to controls [36]. The circulating levels of gelatinases, MMP-2, and MMP-9 have also been well characterized in HF patients. MMP-2 levels were elevated in HFrEF patients when compared to healthy controls and were correlated with LV volume, fractional shortening, and NYHA classifications [37–40]. Furthermore, MMP-2 levels were an independent predictor of mortality in patients with HFrEF [38]. Similarly, clinical studies have reported elevated circulating MMP-9 levels in SHF patients compared to healthy controls [41], but, by contrast, there are also reports that there are no differences between circulating levels of MMP-9 in SHF and control groups [37]. The discrepancies between these studies are not explained by variations in severity, HF etiology, or age. MMP-3 levels were increased in patients with SHF due to dilated cardiomyopathy and were correlated negatively with changes in the LV dimensions and volume, and independently predictive for cardiac events (death and hospitalization) [42]. In addition, MMP-3 levels were increased in patients after acute myocardial infarction who developed CHF [43].

3.3. MMPs in acute coronary syndromes

Acute coronary syndrome (ACS) is a term used for a group of conditions due to decreased blood flow in the coronary arteries such that part of the heart muscle is unable to act properly or dies. ACS is one of the leading causes of cardiovascular death. It includes unstable angina (UA), non-ST segment elevation myocardial infarction (NSTEMI), and myocardial infarction with ST segment elevation (STEMI) [44]. Most cases of ACS are caused by the erosion or rupture of an atherosclerotic plaque, a thickening of the vessel wall in a coronary artery with consequential thrombus formation. ACS is one of the leading causes of cardiovascular death, and early diagnosis of ACS is important because of the improvement in prognosis following timely interventions. It seems that some matrix metalloproteinases are implicated in the pathogenesis of cardiovascular diseases. Inflammatory components also appear to be correlated with development of atherosclerosis and ACS [45].

Hamed and Fattah measured levels of MMP-9 as a potential risk factor in 75 patients with ACS compared to 25 patients with stable angina (SA) and 20 healthy participants. In this study, patients in the SA group had significantly lower MMP-9 levels than those with ACS. Patients with ST-elevated myocardial infarction (MI) had highest MMP-9 levels, while in the control group, the lowest levels of MMP-9 were found. Patients with ACS having poor disease outcome (recurrent ischemic attacks, congestive heart failure, or death) had significantly higher MMP-9 levels. Cutoff value of MMP-9 equal to 3100 pg./mL was able to discriminate MI from unstable angina (UA). The best prognostic utility for MMP-9 was established at 4700 pg./mL [46].

Kai et al. reported that circulating MMP-2 and MMP-9 levels on admission were elevated in patients with acute myocardial infarction (AMI) and UA [47]. Inokubo et al. also reported

that coronary circulation plasma concentrations of MMP-9 were significantly higher in the in-patients with AMI and UA when compared to control subjects. This finding supposes a process of active plaque rupture in acute coronary syndrome [48]. Hirohata et al. and Hojo et al. also observed increased concentrations of plasma MMP-1 and MMP-2 in patients with acute myocardial infarction [49, 50]. In the last ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation, determination of concentrations of high-sensitive troponin T in the short "rule-in"/"rule-out" algorithms is recommended in the early diagnostic of ACS [51]. In the ACS, both MMP-9 and TnT levels are elevated compared to the control group. But, Kobayashi et al. have shown that within 4 h of ACS onset only the plasma level of MMP-9 was markedly increased. By contrast, concentrations of TnT were not significantly altered. In addition, MMP-9 concentrations were significantly higher in patients within first hours of ACS than in patients with late ACS independent of having STEMI or NSTEMI. On the contrary, levels of hs-TnT were significantly lower in patients with early ACS than with late ACS. This study indicates that plasma levels of MMP-9 increase approximately 80 minutes after the onset of ACS and sustain for 24 h thereafter. On the other hand, serum levels of hs-TnT peaked at 12–24 h after ACS onset [52].

The long-term study from Dhillon et al. examined the predictive value for cardiac events (death, readmission with HF, or recurrent MI) of MMP-2, MMP-3, and MMP-9 levels in patients with acute coronary syndrome in comparison to established markers, e.g. N-terminal pro-B-type natriuretic peptide (NT-proBNP) and the Global Registry of Acute Coronary Events (GRACE) score. In this study, MMP-2 and MMP-3 were elevated in patients with fatal outcome compared to survivors but were similar in patients with CHF or MI. MMP-9 levels were similar across study endpoints. Using Cox proportional hazards modeling, MMP-2 demonstrated an independent prediction of death with HR 6.60, along with NT-proBNP (HR 4.62) and the GRACE score (HR 1.03). MMP-3, MMP-9 levels, and log10-troponin I were not predictive for negative outcome. The areas under the receiver operating characteristic curves were 0.60 and 0.58 for MMP-2 and MMP-3, respectively, compared to 0.82 for NT-proBNP and 0.84 for the GRACE score (all statistically significant) for one-year mortality. Kaplan-Meier analysis showed that MMP-2 concentrations in the highest quartile were associated with higher mortality rates ($P = 0.006$, log rank 12.49). MMP-2 and MMP-3 levels revealed a weak association with HF readmission on univariate analysis, which was lost after adjustment for other clinical factors and situations. In this study, none of the MMPs tested predicted onset of MI [53].

As mentioned above, significant portion of regulation components for MMP production in the myocardium after the myocardial infarction is induced by the local proinflammatory cytokines. In an animal model of myocardial injury after AMI, the authors demonstrated that a local increase in TNF-α production in the myocardium is directly responsible for the increased production of local MMP-9 and MMP-2. This is associated with changes in transition of integrin isoforms, which can lead to such aggressive collagen dissolution that causes an acute myocardial rupture. If this process continues without rupture, heart walls become thick and significantly dilated, which leads to decreased function of the ventricle and poor survival. However, deletion of the gene for TNF-α through genetic manipulation in the host

animal leads to a significant reduction in the concentrations of the inflammatory cytokines, which is associated with the reduction in local MMP activation. This results in a significant decrease in the incidence of heart wall rupture and a reduction in the subsequent heart size and development of heart failure with reduced ejection fraction of the LV [2]. Kelly et al. examined the temporal profiles of plasma concentrations of MMP-2 and MMP-9 and their relationship with echocardiographic parameters of left ventricle function and remodeling in humans after acute myocardial infarction. They showed that higher peak concentrations of plasma MMP-9 were associated with the extent of LV remodeling, which led to greater impairment of left ventricular function. In contrast, higher plateau levels of MMP-9 in the days after acute myocardial infarction were associated with a lesser degree of heart remodelation, and relative preservation of ventricular function [54].

3.3.1. Restenosis

The role of MMPs in iatrogenic postprocedural vasculopathy has also attracted a great deal of interest. The development of percutaneous coronary intervention (PCI) has provided a powerful means for treating ischemic heart disease. About 25–40% of patients undergoing PCI without a stent implantation have a recurrence of coronary artery disease symptoms within 6 months because of restenosis at the original site. A combination of events is involved in this pathological process, e.g. the migration and rapid growth of medial vascular smooth muscle cells, local production of chemocytokines, and other biologically active substances with formation of a characteristic lesion of fibrocellular intimal hyperplasia.

Hojo et al. investigated changes in MMP-2 levels in the coronary circulation after PCI in patients with angina pectoris. Plasma MMP-2 levels in the coronary sinus increased significantly 4 h after PCI. A positive correlation between concentrations of MMP-2 in the fourth hour after PCI and *late loss index* measured 6 months after PCI was observed. Authors suggested that excessively raised concentrations of MMPs in dilated coronary arteries after PCI lead to abnormal vascular remodeling and late restenosis by boosting migration of VSMCs and eventual formation of thrombus [55].

3.4. MMPs in atherosclerosis

It is widely accepted that atherosclerosis is promoted by mechanical and/or chemical injury of the vascular endothelium. This is followed by transendothelial migration of circulating monocytes from the circulating blood into the intima, where they become activated and produce a variety of cytokines, growth factors, and other biologically active substances. Formation of an atherosclerotic plaque occurs as a result of cellular migration and local proliferation followed by an accumulation of ECM, lipids, and calcium. Degradation of vascular ECM regulated by MMP-2 promotes smooth muscle cell migration and early plaque development. During the initial period of atherosclerotic plaque development, outward growth produces compensatory enlargement of the artery wall that involves matrix remodeling. Hypertension and aging lead to decrease of arterial compliance, which correlates with gradual accumulation of collagen and loss of elastin. In the latter stages of atherosclerotic process, disruption of the atherosclerotic plaque due to rupture of the fibrous cap or superficial erosion of the endothelium may

occur followed by thrombotic complications. These processes (atherosclerotic plaque rupture and formation of thrombus) depend on excessive ECM degradation. Neovascularization of the atherosclerotic plaque may also play a role in destabilization of the plaque. The angiogenesis within the plaque is influenced and regulated by MMP activity through interactions between proteinases and integrins.

Extreme stage of arterial remodeling due to increased ECM degradation mediated by MMP-2 and MMP-9 is represented as aneurysm formation [56]. Some previous studies showed that lipid-laden macrophages infiltrating human atherosclerotic plaque produce MMP-1 and MMP-3. Culture of these macrophages with fibrous caps of human atherosclerotic plaque leads to MMP-dependent collagen cleaving [57]. Henney et al. found the presence of MMP-3 transcripts in atherosclerotic lesions from coronary arteries, which were localized together with clusters of lipid-laden macrophages in the shoulder areas of the atherosclerotic plaque [58]. Galis et al. reported that normal arteries and atherosclerotic plaque had different origins of MMPs. MMP-2 is secreted by VSMCs in all layers of nonatherosclerotic arteries, whereas in the atherosclerotic lesions, MMP-1, MMP-3, and MMP-9 production is localized to macrophages, VSMCs, and endothelial cells [59]. Some other studies have also reported the expression of several other MMPs including MMP-1, MMP-2, MMP-7, MMP-9, and MMP-12 in the shoulder areas of the plaque [60–62]. The demise of atherosclerotic plaque occurs through structural disruption of the arterial wall, which triggers thrombosis, the cause of occlusion and the majority of acute vascular events. The discovery of strong local MMP overexpression and in situ matrix-degrading activity in the vulnerable shoulders of human atheroma has provided a potential mechanistic insight into the process of plaque destabilization through matrix weakening by MMPs, especially in the vulnerable shoulders [63]. Resident macrophage-derived foam cells, characteristic of unstable plaques, have been identified as a major source of MMPs, including MMP-1, MMP-2, MMP-3, MMP-7, and MMP-associated activity in human and experimental atherosclerotic lesions [64].

It has been suggested that alternative ways or complementary systems for activation of latent MMPs in atherosclerotic plaques may exist. Purified Pro-MMP-2 can be activated in vitro by proteolytical activity of thrombin and this mechanism could provide cell-independent MMP activation at sites of vascular injury [65]. Another proteolytic-activating mechanism of MMP preforms may represent plasminogen cascade. Urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) can also contribute to the development of experimental neointimal lesions after injury and to aortic medial destruction, which was demonstrated in u-PA and PAI-1–null mice [66].

3.5. MMPs in cardiomyopathies

Cardiomyopathies (CMPs) are defined by structural and functional abnormalities of the ventricular myocardium that are unexplained by flow-limiting coronary artery disease or abnormal loading conditions. In the past, this group of diseases has been subdivided into primary CMP, in which the heart is the only involved organ, and secondary forms, where the cardiomyopathy represents a manifestation of other systemic disorders. Hypertrophic cardiomyopathy (HCM) is defined by the presence of increased left ventricular (LV) wall thickness that

is not solely explained by abnormal loading conditions [67], or, in other words, hypertrophic cardiomyopathy (HCM) is defined morphologically as an unexplained hypertrophy in the absence of the reason for such a hypertrophy (e.g. hemodynamic stress). At the histological level is HCM characterized by myocyte disarray, fibrosis, and abnormalities of the intramyocardial small vessels. HCM represents a monogenic disease of the heart with an autosomal dominant way of heritability and different penetrance. Its prevalence in the general population is estimated in the rate of 1/500 [68]. Impaired collagenolysis and an increased deposition of collagen have been seen in patients with HCM. A significant reduction of MMP-1, practically to undetectable levels, was described in the patients with HCM. On the contrary, an increase of MMP-2 and MMP-9 concentration was found in these patients [69]. Described changes in MMP levels result in augmented ECM turnover, characterized by an increase of collagen type I and a shift of collagen I to collagen III production. This shift is not easy to explain because collagen I is patently more rigid than collagen III. This situation could be a compensatory mechanism due to the increase in wall stiffness [70]. Activity of MMP-2 in heart failure with preserved ejection fraction correlates negatively with systolic function of left ventricle and its levels are significantly rising with higher NYHA functional class. In the study of Noji et al., the plasma concentrations of MMP-2, MMP-3, MMP-9, TIMP-1, and TIMP-2 in patients with systolic dysfunction defined as fractional shortening (FS) <25% (group A), linked to HCM, in patients with HCM without systolic dysfunction (FS ≥ 25%; group B), and in healthy control subjects who were age-matched were measured. The concentration of MMP-2 in group A was significantly higher than in group B and the control subjects, whereas there was no significant difference between group B and the control subjects. MMP-2 concentrations significantly increased as the NYHA functional class increased in patients with HCM. MMP-3 and MMP-9 concentrations did not differ among the 3 groups. Both MMP-2 and TIMP-2 correlated significantly with FS and LV dimension, negatively and positively, respectively [71].

The best-known cardiac disease with respect to myocardial MMP activity is dilated cardiomyopathy (DCM). A characteristic feature of the DCM is an increase in left ventricle radius to wall thickness, which increases myocardial wall stress. This leads to further dilation and closes "circulus vitiosus." The etiology of DCM can be divided into ischemic (50–70%) and nonischemic (30–50%), with the latter phenotype including genetic and acquired causes [72]. In Western countries, 20–50% of DCM patients have evidence for familial disease. In animal and human studies in dilated cardiomyopathy, an increase in collagen type I and III production and deposition has been reported, so the collagen type I/type III ratio is constantly increased. As mentioned above, collagen type I is more "rigid" as collagen type III, providing more tensile strength and resulting in a stiffer matrix. As the left ventricle wall gets thicker during the progression of DCM, excessive production of collagen is considered to be an effort to strengthen the heart wall. More core matrix components are overproduced within the DCM-stricken myocardium, including elastin, laminin, and tenascin C. Furthermore, in the DCM heart arise a new complex interplay between various MMPs and TIMPs. This for example includes the MMPs present within the cardiovascular tissue (MMP-1, MMP-2, and MMP-9) and all four known TIMPs. Animal studies and

models show that the enhanced MMP production and protein abundance occur with the initiation of LV dilation, and it might be a very early event in DCM. Progression of DCM is characterized by a decrease in MMP activity, due to an increase in TIMPs production [73]. In a study by Rouet-Benzineb et al., it was demonstrated that MMP-2 and MMP-9 activities were increased in DCM. In this study, MMP-2 levels were significantly increased not only within the myocardial interstitium, but also in cardiocytes. As showed before, MMP-2 may also cleave contractile proteins, e.g. myosin. Thus, increased MMP levels within the myocardium of patients with DCM may have multiple negative consequences that include matrix degradation and proteolytic activation of biologically active signaling molecules and degradation of contractile structures and, finally, directly affect myocyte structure and function [74].

4. Future directions

Ongoing studies targeting receptors for the ECM components have shown potential for new, targeted therapeutics, including several in various stages of clinical trials. Specific ECM proteins interact with cells and play an active role in intercellular signaling to control cell behavior that is critical to the repair or fibrotic process. Effective antifibrotic therapies would be a significant contribution in the treatment of some cardiac diseases as well as many other fibrotic diseases. Strategies reducing overexpression of MMPs in heart diseases may modify the development (or the speed of development) of adverse cardiac remodeling and, e.g., onset of the heart failure after myocardial infarction. This concept has indeed been proved in several basic studies, particularly with the inhibition of MMP-9, which represents one of the major MMPs involved in myocardial remodeling. Most of these studies show improvement in ventricular function and a reduction in ventricular size after administration of the MMP inhibitors [75]. Unfortunately, administration of various MMP inhibitors led to adverse events and number of leading candidates for the therapy have been withdrawn from development for this indication because of onset of fibromyalgia side effects in earlier trials attempting to decrease metastasis in cancer. Pharmacologic strategies affecting upstream signaling cascades involved in MMP transcription and regulation can participate in the process of our understanding of the complex myocardial remodeling and the specific role of MMPs and TIMPs. For example, the use of cytokine inhibition in biological therapy (such as administration of tumor necrosis factor-α neutralizing proteins) may prove to be useful pharmacological tools in order to identify the signaling pathways obligatory for MMP species induction [76].

On the other hand, many of the currently used treatment strategies may already partially affect MMP activation pathways as part of their "modus operandi." For example, part of the benefit of treatments such as acetylsalicylic acid or statins may decrease the cytokine and inflammatory response and so limit the bioactivity of MMPs.

Tissue engineering may open new avenues to create intelligent scaffolds to support regeneration of diseased or damaged tissue.

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