Cellular Mechanisms of Tissue Fibrosis. 2. Contributory pathways leading to myocardial fibrosis: moving beyond collagen expression

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Submitted 1 November 2012; accepted in final form 20 November 2012

Goldsmith EC, Bradshaw AD, Spinale FG. Cellular Mechanisms of Tissue Fibrosis. 2. Contributory pathways leading to myocardial fibrosis: moving beyond collagen expression. Am J Physiol Cell Physiol 304: C393–C402, 2013. First published November 21, 2012; doi:10.1152/ajpcell.00347.2012.—While the term “fibrosis” can be misleading in terms of the complex patterns and processes of myocardial extracellular matrix (ECM) remodeling, fibrillar collagen accumulation is a common consequence of relevant pathophysiological stimuli, such as pressure overload (PO) and myocardial infarction (MI). Fibrillar collagen accumulation in both PO and MI is predicated on a number of diverse cellular and extracellular events, which include changes in fibroblast phenotype (transdifferentiation), post-translational processing and assembly, and finally, degradation. The expansion of a population of transformed fibroblasts/myofibroblasts is a significant cellular event with respect to ECM remodeling in both PO and MI. The concept that this cellular expansion within the myocardial ECM may be due, at least in part, to endothelial-mesenchymal transformation and thereby not dissimilar to events observed in cancer progression holds intriguing future possibilities. Studies regarding determinants of procollagen processing, such as procollagen C-endopeptidase enhancer (PCOLCE), and collagen assembly, such as the secreted protein acidic and rich in cysteine (SPARC), have identified potential new targets for modifying the fibrotic response in both PO and MI. Finally, the transmembrane matrix metalloproteinases, such as MMP-14, underscore the diversity and complexity of this ECM proteolytic family as this protease can degrade the ECM as well as induce a profibrotic response. The growing recognition that the myocardial ECM is a dynamic entity containing a diversity of matrix-cellular and nonstructural proteins as well as proteases and that the fibrillar collagens can change in structure and content in a rapid temporal fashion has opened up new avenues for modulating what was once considered an irreversible event - myocardial fibrosis.

fibril; collagen; myocardium; extracellular matrix

THE CLINICAL PRESENTATION of heart failure (HF), which afflicts millions of patients annually, is generally that of a common set of clinical signs and symptoms, whereby fundamental defects in left ventricular (LV) function invariably underlie this clinical syndrome. The structural milestones that contribute to changes in LV function and therefore the development and progression of HF include changes in the structure, composition, and geometry of the LV myocardium, which has been generally termed LV remodeling. There are fairly distinct patterns of LV remodeling that occur and are dependent on the initial pathophysiological stimulus, but once initiated, LV remodeling is an important predictor for the development and progression of HF. LV remodeling entails changes in the structure and function of the cardiocyte, the vascular compartment, and extracellular matrix (ECM), whereby changes within all of these entities occur as a continuum during the initiation and progression to HF. Most certainly, fundamental defects in cardiocyte function and viability as well as vascular structure play critical roles in the development and progression to HF. However, there is growing recognition that the ECM mediates both mechanical and biological signals that contribute to this process (26, 39, 40, 45, 48, 73). For example, the ECM provides the critical interface for force transmission and alignment of myocardial fascicles and also provides the substrate for transmembrane adhesion of cardiocytes (4, 38, 39, 44, 47, 71). Thus, a loss of normal ECM structure and function can directly alter transduction of contractile force and intracellular signaling of cardiocytes, which in turn will change LV systolic function. In contrast, excessive ECM accumulation can directly alter myocardial passive stiffness properties, which will directly affect LV diastolic function (1, 21, 43, 90). In certain instances, significant heterogeneity in ECM remodeling occurs whereby a loss of normal ECM structure and function can be accompanied by abnormal ECM accumulation, which can impair both LV systolic and diastolic function (28, 58, 74, 92). The classical approach for evaluating the myocardial ECM has been that of a binary evaluation, whereby histopathological scoring for fibrillar collagen was judged either as apparently “normal” or “increased” in relative content, whereby the latter has been termed myocardial “fibrosis.” These past studies, while providing valuable insight into gross patterns of LV remodeling in terms of fibrillar collagen accumulation, also tended to promulgate the concept that the myocardial ECM is a static structure with a unidirectional response to a pathological stress. However, it is now recognized that the ECM is a dynamic entity containing a diversity of structural and non-structural proteins and molecules. Through the use of transgenic constructs, imaging modalities, and greater attention to posttranscriptional and posttranslational regulatory pathways, a greater insight into the highly complex and tightly regulated myocardial ECM has become appreciated (1, 4, 21, 25, 28, 38, 39, 43, 44, 47, 58, 71, 74, 90). The overall goal of this themed review is to more closely examine a few of the newer insights regarding specific cellular and posttranslational pathways relevant to the LV myocardial ECM remodeling process, as it relates to the generalized concept of LV myocardial fibrosis. First, generalized patterns of LV remodeling as it relates to
ECM accumulation, such as fibrosis, will be briefly described. Second, a reevaluation of the most numerous cell types within the myocardium critical to ECM homeostasis, the fibroblast in relation to LV remodeling, will be discussed. Third, some important posttranslational processing steps in terms of collagen fibril assembly and stability and the relation to LV remodeling will be defined. Fourth, ECM proteolysis will be briefly presented as a dynamic and ongoing process and will also directly determine net ECM accumulation. The ultimate goal of this themed review is to recognize the importance of evaluating a number of posttranslational determinants of collagen biosynthesis/degradation and outline some future directions that would be relevant to a more comprehensive view of the ECM in the context of LV remodeling and HF.

Glossary

- ADAMTS: A disintegrin and metalloproteinase with thrombospondin motifs
- ANG II: angiotensin II
- BMP: bone morphogenetic protein
- DDR2: discoidin domain receptor 2
- ECM: extracellular matrix
- EGFP: enhanced green fluorescent protein
- EMT: epithelial-mesenchymal transformation
- EndoEMT: endothelial cell EMT
- FSP1: fibroblast-specific protein 1
- HF: heart failure
- LV: left ventricular
- MI: myocardial infarction
- MMP: matrix metalloproteinase
- mRNA: messenger RNA
- PAB: pulmonary artery banding
- PCOLCE: procollagen C-endopeptidase enhancer
- PO: pressure overload
- sFRP: secreted frizzled-related protein
- shRNA: small hairpin RNA
- SMA: smooth muscle actin
- SPARC: secreted protein acidic and rich in cysteine
- TAC: transverse aortic constriction
- TGF: transforming growth factor
- TSP2: thrombospondin 2
- WT: wild type
- Wt1: Wilm’s Tumor 1

LV Remodeling and the Extracellular Matrix

To provide a foundation for the focus on the ECM and this themed review on myocardial fibrosis, generalities regarding the LV remodeling process that can lead to HF include the following: 1) the pressure overloaded (PO) myocardium, such as that with hypertension or aortic stenosis, which causes LV hypertrophy and can give rise to increased collagen accumulation (i.e., fibrosis); and 2) the injured myocardium, most notably that of myocardial infarction (MI), whereby a heterogeneous remodeling process can occur simultaneously within the LV myocardium, giving rise to myocardial hypertrophy and fibrosis within the remote viable region, as well as mural wall thinning, expansion, and loss of normal collagen matrix structure and function within the MI region. While there are a number of other relevant LV remodeling processes that can lead to HF (i.e., cardiomyopathies, volume overload), PO and MI can serve as prototypical of LV remodeling processes whereby increased ECM accumulation, which has been termed fibrosis, invariably occurs. In PO, the fundamental stimulus for ECM growth and accumulation is the same for cardiocyte growth: increased LV afterload. While initially considered an adaptive process, the LV hypertrophy with PO will cause excessive fibrillar collagen accumulation as well as a number of other ECM proteins and ultimately impair LV filling, resulting in diastolic dysfunction and eventually HF. Following MI, a loss of cardiocyte mass replaced by an unstable ECM, the infarct scar, can eventually lead to LV remodeling, notably characterized by LV dilation and eventually pump dysfunction. The region remote from the MI can undergo remodeling not dissimilar to PO whereby there is growth of both the cellular and extracellular compartments, and the increased ECM can be paralleled by increased regional myocardial stiffness. A brief summary of the LV remodeling phenotype, which occurs in PO or MI, is shown in Table 1. The ECM contains important structural fibrillar collagens, such as collagen I and III, but there is also a large portfolio of other collagen types that can span the interstitial space and comprise the basement membrane (37, 91). While the fibrillar collagens have been a primary focus for structure-function studies in regards to the myocardial ECM, it should be recognized that this is but a small fraction of diverse molecules constituting this interstitial entity. Proteoglycans, such as chondroitin sulfate, dermatan sulfate, and perlecan are only a few examples of a class of complex molecules contained within the ECM. Glycoproteins, such as laminin and fibronectin, are critical constituents of the ECM and basement membrane facilitating cell-ECM adhesion through interaction with the transmembrane ECM receptors, the integrins (71). Many of these ECM constituents are synthesized primarily by the myocardial fibroblast, and yet this cell type is often not rigorously evaluated in the context of LV remodeling and the progression to HF. As detailed in a subsequent section, significant changes in fibroblast growth, proliferation, and functional phenotype occur in the context of both PO and MI, which directly hold relevance to the directionality of the ECM remodeling process. Most intriguing is the concept that there is a population expansion of “transformed” fibroblasts within the myocardial interstitium that occurs following a pathophysiological stimulus. While the measurement of fibrillar collagen mRNA levels can be used as one index of the directionality of ECM remodeling, this measurement alone is not conclusive. Specifically, processing of the newly secreted procollagen molecules is essential for the formation of stable collagen fibrils and ultimately collagen accumulation within the myocardium. As such, some of the newer aspects and determinants of procollagen processing, such as procollagen C-endopeptidase enhancer (PCOLCE) and secreted frizzled-related protein (sFRP), will be discussed in the context of myocardial ECM remodeling. There is a growing list of macromolecular proteins secreted into the ECM with a diversity of biological functions, and several of these directly influence collagen stability and assembly. A prototypical example will be briefly presented, which is the secreted protein acidic and rich in cysteine (SPARC). Finally, a highly potent family of ECM degradative enzymes exists within the myocardium, the matrix metalloproteinases (MMPs). These MMPs can proteolytically process newly formed collagen as well as mature collagen, and as such, play a critical role in overall ECM...
Table 1. Generalized features of LV remodeling with either pressure overload or myocardial infarction in terms of LV structure, function, and extracellular matrix

<table>
<thead>
<tr>
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<th>Pressure Overload</th>
<th>Myocardial Infarction</th>
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<tbody>
<tr>
<td>LV remodeling phenotype</td>
<td>Myocardial hypertrophy</td>
<td>Thinning and expansion of MI region and myocardial hypertrophy in the remote, viable region</td>
</tr>
<tr>
<td>LV functional phenotype</td>
<td>Abnormal diastolic function primarily characterized by increased myocardial stiffness</td>
<td>LV dilation and systolic dysfunction primarily characterized by infarct expansion process</td>
</tr>
<tr>
<td>Fibroblast response</td>
<td>Proliferation of fibroblasts and transdifferentiation of fibroblasts primarily characterized by enhanced profibrotic signaling</td>
<td>Fibroblast transdifferentiation within the MI region primarily characterized as an amplification of both matrix degradation and synthesis pathways, and fibroblast proliferation within the remote region primarily characterized by enhanced profibrotic signaling</td>
</tr>
<tr>
<td>Matrix remodeling response</td>
<td>Enhanced fibrillar collagen synthesis coupled with reduced degradation and turnover results in myocardial collagen accumulation—i.e., fibrosis</td>
<td>Continuous fibrillar collagen turnover within the MI region and amplified degradation pathways results in increased collagen accumulation but an overall abnormal matrix in terms of structure and function. Increased fibrillar collagen synthesis within the remote region (not dissimilar to PO) results in increased collagen accumulation—i.e., fibrosis.</td>
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LV, left ventricular; PO, pressure overload; MI, myocardial infarction.

Fibroblasts have long been recognized as the most numerous cell types in the rodent myocardium, and recent work has shown that the number of fibroblasts is dynamic, changing during development and postnatal growth with increased hemodynamic load (6, 59, 96). Following development and under normal physiological conditions, it appears that myocardial fibroblast turnover is limited, and very few myofibroblasts exist within the normal myocardium (7). However, with a pathophysiological stimulus such as PO, fibroblast proliferation occurs (5, 45, 76). For example, PO in rats caused fibroblast proliferation, particularly in the perivascular space within 72 h following PO induction, which was then followed by increased fibroblast proliferation within the entire myocardial ECM (45). In this study, the proliferating fibroblasts coted with α-smooth muscle actin (α-SMA), indicating the presence of myofibroblasts. In a mouse PO model, α-SMA-positive myofibroblasts were observed 3 days post-PO, and the number increased over a 28-day observation period (93). Lineage tracing studies using a Tie1Cre mouse subjected to PO demonstrated that around 25% of fibroblasts and close to 75% of myofibroblasts within the myocardium were derived from a labeled endothelial cell precursor, suggesting that these cells arose from EndoEMT (97). Thus, there is a rapid proliferation of fibroblasts as well as a phenotype shift to myofibroblasts that contributed to fibroblast-myofibroblast populations within the MI. In addition, myofibroblasts also play a prominent role in the fibrosis associated with PO. In vitro studies have demonstrated that cardiac fibroblasts in response to mechanical loading rapidly differentiated into myofibroblasts (89). In vivo, differentiated myofibroblasts can arise from bone marrow-derived cells in addition to differentiation of resident, myocardial fibroblasts (23). As was observed in the MI model, the myofibroblast can also be viewed as a dynamic population within the PO myocardium. In a right ventricular PO model, myofibroblast density increased within the first week after the PO stimulus and had decreased by 2 wk, which paralleled the rate of ECM accumulation (49). Fibroblasts isolated from a similar PO model demonstrated higher collagen deposition in vitro in comparison to non-PO fibroblasts, thereby demonstrating that a phenotype shift had occurred in terms of ECM synthesis (64).

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following a PO stimulus. A more robust shift in the number of fibroblasts/myofibroblasts occurs following MI and is likely due to a combination of proliferation/differentiation of existing fibroblasts, infiltration of stem cells, and EndoEMT (19, 87). A summary of potential sources and cell types that may give rise to fibroblast proliferation and transdifferentiation is shown in Table 2. For example, it has been shown in a mouse MI model that fibroblasts within the MI region differentiate from resident CD44+ mesenchymal stem cells within 3 days post-MI; however, CD44+/α-SMA-positive cells were not readily observed until 7 days post-MI (16). Using a bone marrow reporter mouse construct, nearly 25% of fibroblasts and almost 60% of myofibroblasts identified within the MI region were positive for the reporter protein, suggesting these cells originated from the bone marrow cells (57). Thus, there appears to be a significant expansion of nonresident fibroblasts following MI, which may be the result of an EndoEMT type process involving a nondifferentiated population of stem cells. However, whether and to what degree this process contributes to the overall changes in ECM architecture following MI require further study.

Cardiac myofibroblasts represent an activated phenotype of the fibroblast, associated with a number of functions including increased collagen gel contraction, increased collagen deposition, and ultimately myocardial fibrosis (24, 51, 52). While myocardial fibroblasts can be distinguished by their expression of discoidin domain receptor 2 (DDR2), αvβ3 integrin, vimentin, and Thy-1, the most commonly used marker to identify myofibroblasts is the expression of α-SMA, a contractile protein normally associated with smooth muscle cells that imparts an enhanced contractile phenotype to the activated fibroblast (12, 27, 31, 36). In addition to α-SMA, desmin, tropomyosin-1, and non-muscle myosin-heavy chain B are also expressed by myofibroblasts (27, 66). However, previous studies have shown that even after differentiation into myofibroblasts, expression of some fibroblast-associated proteins, such as αvβ3 integrin, DDR2, and Thy-1, persists (12, 36, 67). Isolated myocardial fibroblasts plated on rigid culture substrates have been shown to rapidly convert to a myofibroblastic phenotype, evidenced by increased expression of both α-SMA and embryonic smooth muscle myosin (67). Examination of the MI region has revealed positive staining for α-SMA and DDR2, suggesting that myofibroblasts in vitro and in vivo express the same protein markers (67). However, it can be difficult to quantify and localize fibroblasts versus myofibroblasts in terms of these markers. The complexity of expression of these different proteins and markers of myofibroblasts can be appreciated when colocalization of actin, collagen type I, and DDR2 is performed (Fig. 1). As noted above, there are several current markers used to distinguish fibroblasts/myofibroblasts from other cell types within the heart and far fewer that can be used to discern them from one another. This is an important area of further research as the transdifferentiation of fibroblasts, either from a pool of endogenous cells or from a margination of exogenous cells followed by EndoEMT and expansion, could be an important cellular target for the regulation of myocardial fibrosis and adverse ECM remodeling in both PO and MI.

One fairly recent observation is that there may be an intermediate stage in the differentiation of fibroblasts into myofibroblasts: the proto-myofibroblast (81). Proto-myofibroblasts do not express α-SMA and are characterized by the presence of stress fibers in response to the development of mechanical tension, the formation of focal adhesions, and expression of the ED-A splice variant of fibronectin; however, very little else is known about this particular cell type within the myocardium. It has been reported that P311 [also known as PTZ17, an 8-kDa protein containing several PEST-like domains; structural motifs rich in proline, glutamic acid (E), serine, and threonine that are targets for protein degradation machinery and are typically found in proteins with short half-lives] could induce myofibroblast differentiation in mouse NIH 3T3 fibroblasts (63). Immunohistochemistry demonstrated that P311 was expressed by proto-myofibroblasts and myofibroblasts in vivo but not expressed in fibroblasts (63). More recent studies examining the role of P311 in human fibroblasts derived from hypertrophic scars suggest that this protein stimulates myofibroblast differentiation associated with increased profibrotic signaling pathways, notably transforming growth factor (TGF) (79). While remaining speculative, these observations suggest the intriguing possibility that the proto-myofibroblast may represent the checkpoint in the evolution of the myofibroblast phenotype, and proteins such as P311 could regulate the profibrotic potential of this cellular phenotype.

Critical Steps in Collagen Biosynthesis and Relevance to LV Remodeling

A generalized schematic that highlights just some of the important posttranslational steps in fibrillar collagen processing is shown in Fig. 1, and it serves to underscore not only the complexity of this process but also the potential targets for modulation of collagen accumulation within the myocardium. Fibrillar collagens are secreted as triple helical procollagen molecules with NH2 and COOH-terminal propeptides that must be removed prior to being deposited into stable, mature collagen within the ECM (65). Members of the ADAMTS family, ADAMTS 2 and 3, cleave the NH2-terminal propeptide, whereas BMP-1 (and related family members) clips off the COOH-terminal propeptide (18, 41). Enhancers of BMP-1 activity with regard to fibrillar procollagens expressed in the myocardium include PCOLCE 1 and 2 as well as sFRP 2. In the case of PCOLCE 1 and 2, these proteins bind to the C-propeptide and increase the efficiency of C-propeptide cleavage up to 20-fold (83). Release of the COOH-terminal propeptide is considered a critical event in converting soluble procollagen to insoluble fibrillar collagen. Interestingly, the LV

### Table 2. Cardiac fibroblast precursors

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Disease Model</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Bone marrow-derived stem cells</td>
<td>MI, PO</td>
<td>24, 57</td>
</tr>
<tr>
<td>Endogenous mesenchymal stem cells</td>
<td>MI</td>
<td>16, 19</td>
</tr>
<tr>
<td>Endothelium (EndoEMT)</td>
<td>MI, PO</td>
<td>87, 96</td>
</tr>
<tr>
<td>Epicardium</td>
<td>MI</td>
<td>85, 87</td>
</tr>
<tr>
<td>Epithelium (EMT)</td>
<td>MI</td>
<td>24</td>
</tr>
<tr>
<td>Myeloid-derived fibroblasts</td>
<td>MI</td>
<td>19</td>
</tr>
<tr>
<td>Pericytes</td>
<td>Cardiac development</td>
<td>73, 73</td>
</tr>
<tr>
<td>Monocytes</td>
<td>MI</td>
<td>19</td>
</tr>
<tr>
<td>Resident fibroblasts</td>
<td>MI, PO</td>
<td>24, 94</td>
</tr>
</tbody>
</table>

A number of cell types and sources likely give rise to fibroblast transdifferentiation and proliferation, which in turn will contribute to an advanced myocardial matrix structure and function with PO or MI. EndoEMT, endothelial-mesenchymal transformation; EMT, epithelial-mesenchymal transformation.
Myocardium was found to have the highest levels of expression of PCOLCE 1 and 2 in comparison to other tissues, including lung, liver, and skeletal muscle (75). This observation would imply that regulation of procollagen processing by PCOLCE is particularly relevant within the myocardium and is discussed in a subsequent paragraph.

LV myocardial fibrosis in response to PO occurs over a time scale distinct from that of myocyte hypertrophy. In other words, past studies have shown that the induction of the PO stimulus first causes an adaptive myocyte growth response, which is subsequently followed by measurable changes in collagen content (4). For example, using a right ventricular PO model, it was demonstrated that robust myocyte hypertrophy occurred within the initial 2 weeks following PO, but observable differences in collagen accumulation were not detected until myocyte hypertrophic growth had plateaued (4). In other studies, a robust increase in mRNA encoding fibrillar collagens occurs early following the induction of PO but actually declines prior to a quantifiable change in myocardial fibrillar collagen (86). The proportion of newly synthesized procollagen that is degraded prior to ECM incorporation is ~50% in adult myocardium, as detected by measuring incorporation of
the matricellular proteins that may play an important role in significantly to critical steps in ECM structural assembly. Some of identified within the myocardium and can contribute significantly to the adverse ECM remodeling, which is apparent that sFRP2 can modify myocardial collagen content in a manner relevant to the adverse ECM remodeling, which was detected at the cell surface/pericellular locations in comparison to wild-type fibroblasts (34). In this past study, increased amounts of fully processed collagen type I was localized to the cell-ECM interface of SPARC-null fibroblasts, indicative of increased procollagen processing efficiency. These results would suggest that the absence of SPARC would facilitate the fully processed collagen type I to be incorporated into the mature, more insoluble ECM. However, in vivo findings do not support this supposition and surprisingly identified the opposite effect (13). Specifically in a murine model of PO, genetic deletion of SPARC significantly attenuated collagen fibril formation and overall collagen accumulation (13). In addition, impaired collagen maturation and MI scar formation was observed in SPARC-null mice, which was associated with higher post-MI mortality rates compared with wild-type mice (68). These results support the hypothesis that the absence of SPARC greatly reduces the relative rate and efficiency of collagen maturation within the myocardium. These observations hold relevance for future basic and translational studies with respect to regulation of posttranslational ECM processing, but they also underscore the significant difference in terms of ECM accumulation regarding PO and MI. Specifically, inhibition of SPARC in the context of PO may alter the rate and magnitude of myocardial collagen accumulation, which in turn would potentially yield favorable effects on LV diastolic function and delay the progression to HF. In contrast, inhibition of SPARC may cause inadequate fibrillar collagen assembly within the critical wound healing phase post-MI with adverse consequences on LV remodeling and function. Thus, it will be important in future studies to evaluate these critical posttranslational ECM pathways that regulate “fibrosis” in a temporal and regional context. Furthermore, modification of the relative “maturity” and stability of the ECM, the fibrillar collagens in particular in terms of posttranslational processing, will also alter the vulnerability to proteolysis, which is outlined in the next paragraph.

Matrix Proteolytic Enzymes Facilitate Both ECM Instability and Fibrosis

There are now approximately 23 MMP types expressed within humans, and the distribution, functionality, and substrates are highly diverse as discussed in several broader reviews (11, 17, 33, 40). After approximately two decades of research, there is no question that changes in MMP type and activity directly influence the myocardial ECM and hold particular relevance to LV remodeling processes, such as PO and MI. What is becoming more recently appreciated, however, is that certain MMPs not only facilitate collagen degradation but can also process profibrotic signaling molecules. Thus, MMPs likely contribute to the degradation of relatively normal ECM structure as well as facilitate the deposition of a more “fibrotic” and dysfunctional myocardial ECM. The MMPs can be broadly classified into the “collagenases,” such as MMP-1, MMP-13, and MMP-8; the “gelatinases” would include MMP-2 and MMP-9; the “stromelysins/matrilysins” would...
include MMP-3 and MMP-7; and the “membrane type MMPs” would include MMP-14. This is hardly the exhaustive list of MMP types that are likely expressed within the mammalian myocardium. For the purposes of highlighting the duality and diversity of the MMP family, MMP-14 will be discussed. The pathways by which MMP-14 contributes to adverse ECM remodeling likely include facilitating proteolysis of interstitial molecules directly (such as integrins), amplification of active MMP-2 causing ECM instability and abnormal architecture, as well as through enhancing profibrotic signaling pathways (2, 32, 48, 74, 80). Of more recent discovery is that an important proteolytic relationship likely exists between MMP-14 and the subsequent activation of the profibrotic signaling molecule TGF, which would hold particular relevance in the context of myocardial fibrosis (48, 80). In animal models of PO, an early and sustained induction of MMP-14 has been identified (95, 98). For example, increased MMP-14 promoter activity and subsequently MMP-14 proteolytic activity has been reported following the induction of PO in mice (98). Moreover, these studies have identified an association between changes in myocardial ECM remodeling, notably increased collagen accumulation to that of MMP-14 induction. In murine models of MI, increased levels of MMP-14 were identified within both the MI and the remote regions but were associated with significantly different effects (74). Precisely, using an MMP-14-specific fluorogenic substrate, it was demonstrated that increased MMP-14 activity occurred within the MI region and was associated with increased MMP-2 activation, which in turn would cause ECM instability. On the other hand, increased MMP-14 activity within the remote region was associated with heightened indices of TGF signaling and was associated with increased collagen accumulation. These past observations underscore that certain MMP types do not only degrade the ECM, but likely play a much larger and diverse role in ECM remodeling. Moreover, assessing MMP expression alone may be insufficient in providing insight into the type of ECM remodeling process that is occurring in either PO or MI.

**Summation and Future Directions**

The growing recognition that the myocardial ECM is a dynamic entity that contains biological signaling molecules, a diversity of matricellular and nonstructural proteins, and that the fibrillar collagens can change in structure and content in a rapid temporal fashion has opened up new avenues for modulating what was once considered an irreversible event—myocardial fibrosis. Using both metabolic and racemization/isomerization studies, it appears that fibrillar collagen turnover within the LV myocardium is one of the highest of any organ, and thus challenges the historical concept that myocardial fibrosis is a dormant, end-stage process (29, 56). While the term “fibrosis” can be misleading in terms of the complex patterns and processes of ECM remodeling within the myocardium, there is no question that fibrillar collagen accumulation is a common consequence of two relevant pathophysiological stimuli: PO and MI. The fact that transdifferentiation of fibroblasts to a myofibroblast phenotype occurs in both PO and MI, and may be the result of EndoEMT, holds a number of intriguing future directions. First, the novel insights of this transdifferentiation process that have been provided from the large body of cancer research may be applicable to adverse ECM remodeling process with PO and MI. Second, the possibility that the proliferation of a specific stem cell-derived population of myofibroblasts may occur in these cardiac disease states may afford a number of interventional possibilities. Third, since myofibroblasts that populate the MI region can persist for months to years following the initial MI, this certainly affords an important cellular target in terms of therapeutics (77). However, a number of obstacles must be overcome and will need to be addressed in future studies. These would include improved methods for identifying fibroblast-myofibroblast phenotype, understanding the transdifferentiation process to a much greater degree, particularly in terms of a “transitional state” of fibroblast-myofibroblast transdifferentiation, and more specific gene targeting tools (i.e., fibroblast- vs. myofibroblast-specific promoters). While there are a number of profibrotic signaling molecules relevant to myocardial ECM remodeling, most certainly TGF is considered a predominant pathway. More importantly, however, interruption of TGF signaling directly or indirectly, such as through regulation of P311, may afford an opportunity to modify fibroblast transdifferentiation and in turn, the course of myocardial ECM remodeling. It is becoming not only clear that myofibroblasts

### Table 3. Matricellular proteins expressed within the myocardium and potentially relevant collagen matrix biosynthesis

<table>
<thead>
<tr>
<th>Transgenic Null Phenotype</th>
<th>Effects on Collagen Fibril Morphology</th>
<th>Cardiac ECM Homeostasis</th>
<th>ECM Remodeling Post-MI or PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPARC</td>
<td>Decreased dermal collagen fibril diameter (14)</td>
<td>Reduced collagen content in SPARC-null hearts (13)</td>
<td>MI: increased incidence of rupture, decreased collagen content and fiber maturity (68)</td>
</tr>
<tr>
<td>Periostin</td>
<td>Decreased dermal collagen fibril diameter, reduced collagen cross-links (61)</td>
<td>MI: increased incidence of rupture, reduced collagen fibril diameter, reduced collagen cross-links (62, 72)</td>
<td>PO: reduced collagen content (62)</td>
</tr>
<tr>
<td>Thrombospondin 2</td>
<td>Increased dermal collagen fibril diameters, aberrant fibrils (46)</td>
<td>Reduced collagen content with aging (78)</td>
<td>MI: increased incidence of rupture (69)</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Decreased dermal collagen fibril diameters in wounds (50)</td>
<td>No difference in collagen content, disrupted fibrillar collagen weaves (82)</td>
<td>PO: increased rupture in response to ANG II infusion, decreased fibrosis (70)</td>
</tr>
<tr>
<td>Tenascin C</td>
<td>None reported</td>
<td>No differences detected (60)</td>
<td>MI: decreased collagen content in border zone (60)</td>
</tr>
<tr>
<td>Tenascin X</td>
<td>Reduced dermal fibril density (53)</td>
<td>Not characterized</td>
<td>Not characterized</td>
</tr>
</tbody>
</table>

ECM, extracellular matrix; SPARC, secreted protein acidic and rich in cysteine. Reference nos. in parentheses.
represent a cellular target for modifying the ECM remodeling process, but also this cell transdifferentiation process can cause changes in posttranslational collagen processing (64). This set of posttranslational events that yield a mature and stable collagen matrix within the ECM is highly orchestrated (Fig. 1). Targeting key steps/proteins in this posttranslational process that are robustly expressed within the myocardium, such as PCOLCE 1 and 2, as well as matricellular proteins, such as SPARC in the context of PO and MI, may be a novel approach in favorably modifying the ECM and as a result slow the progression of adverse LV remodeling (75). Since a number of these ECM posttranslational processing steps are cell surface associated, transmembrane proteases would hold particular relevance in terms of degradation of the immature, poorly processed collagen. The transmembrane protease MMP-14 is a prototypical example of the tight interaction between profibrotic and degradative pathways. For example, MMP-14 degrades fibrillar collagens directly as well as through the activation of other MMPs, which in turn would alter the turnover and stability of the myocardial ECM. On the other hand, MMP-14 facilitates TGF signaling within the ECM and in turn would amplify a profibrotic signaling cascade. Targeting MMP-14, therefore, may provide an avenue to simultaneously prevent the loss of normal ECM structure and abnormal ECM accumulation, both of which occur in the context of PO and MI. These are but a few examples of the highly diverse and dynamic nature of the myocardial ECM and underscore the need for a more thorough understanding of cellular and extracellular pathways that contribute to the changes in myocardial collagen assembly and accumulation, which in turn will likely yield important new therapeutic targets.

ACKNOWLEDGMENTS

The authors express appreciation to Ashley Sapp, University of South Carolina School of Medicine, for editorial assistance.

GRANTS

This work was supported by National Institutes of Health Grants HL-057952, HL-089944, HL-095608, HL-111090, HL-097214, and HL-094517 and by Merit Awards (5I01BX000168 and 1I01BX001385) from the Veterans’ Affairs Health Administration.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

E.C.G. and F.G.S. prepared the figure; E.C.G., A.D.B., and F.G.S. drafted the manuscript; E.C.G., A.D.B., and F.G.S. edited and revised the manuscript; F.G.S. approved the final version of the manuscript.

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