

This Review is in a thematic series on **The Myocardial Extracellular Matrix: An Ever-Changing and Diverse Entity**, which includes the following articles:

Translating Koch's Postulates to Identify Matrix Metalloproteinase Roles in Postmyocardial Infarction Remodeling: Cardiac Metalloproteinase Actions (CarMA) Postulates [*Circ Res.* 2014;114:860–871]

**Myocardial Extracellular Matrix: An Ever-Changing and Diverse Entity**

Matrix as an Interstitial Transport System

Molecular Imaging of the Cardiac Extracellular Matrix

Dynamic Changes in Myocardial Matrix and Relevance to Disease: Translational Perspectives

*Francis Spinale, Guest Editor*

## Myocardial Extracellular Matrix An Ever-Changing and Diverse Entity

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**Abstract:** The cardiac extracellular matrix (ECM) is a complex architectural network consisting of structural and nonstructural proteins, creating strength and plasticity. The nonstructural compartment of the ECM houses a variety of proteins, which are vital for ECM plasticity, and can be divided into 3 major groups: glycoproteins, proteoglycans, and glycosaminoglycans. The common denominator for these groups is glycosylation, which refers to the decoration of proteins or lipids with sugars. This review will discuss the fundamental role of the matrix in cardiac development, homeostasis, and remodeling, from a glycobiology point of view. Glycoproteins (eg, thrombospondins, secreted protein acidic and rich in cysteine, tenascins), proteoglycans (eg, versican, syndecans, biglycan), and glycosaminoglycans (eg, hyaluronan, heparan sulfate) are upregulated on cardiac injury and regulate key processes in the remodeling myocardium such as inflammation, fibrosis, and angiogenesis. Albeit some parallels can be made regarding the processes these proteins are involved in, their specific functions are extremely diverse. In fact, under varying conditions, individual proteins can even have opposing functions, making spatiotemporal contribution of these proteins in the rearrangement of multifaceted ECM very hard to grasp. Alterations of protein characteristics by the addition of sugars may explain the immense, yet tightly regulated, variability of the remodeling cardiac matrix. Understanding the role of glycosylation in altering the ultimate function of glycoproteins, proteoglycans, and glycosaminoglycans in the myocardium may lead to the development of new biochemical structures or compounds with great therapeutic potential for patients with heart disease. (*Circ Res.* 2014;114:872-888.)

**Key Words:** glycoproteins ■ glycosaminoglycans ■ glycosylation ■ proteoglycans

Cardiac extracellular matrix (ECM) is a complex meshwork of fibers comprised of matrix proteins in which cardiac myocytes, fibroblasts, leukocytes, and cardiac vascular cells reside. Beside its structural role giving strength and support to cardiac cells, cardiac ECM also has important nonstructural properties by accommodating multiple proteins with growth factor- and cell receptor-binding properties. The present review will describe in detail the central involvement

of nonstructural proteins in cardiac development and cardiac remodeling. Our focus will mainly be on glycosylation, the decoration of proteins with added sugars that results in diversification of protein functionality, creating proteoglycans and glycoproteins, such as the well-known matricellular proteins.<sup>1,2</sup> To facilitate the understanding of functional diversity that added sugars contribute to cardiac ECM, we will briefly describe some basic biochemical concepts regarding their

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Nonstandard Abbreviation and Acronyms	
CS	chondroitin sulfate
DS	dermatan sulfate
ECM	extracellular matrix
ER	endoplasmic reticulum
FGF	fibroblast growth factor
GAG	glycosaminoglycan
HS	heparan sulfate
MI	myocardial infarction
MMP	matrix metalloprotease
OPN	osteopontin
SLRP	small leucine-rich proteoglycan
SPARC	secreted protein acidic and rich in cysteine
TGF	transforming growth factor
TLR	toll-like receptor
TSP	thrombospondin

biosynthesis and structure. Subsequently, we will discuss established functions and speculate on probable functions of essential sugars and of the most important extracellular proteoglycans and glycoproteins (Figure 1).

### Orchestrating the Ever-Changing Appearance of Cardiac ECM

Cardiac remodeling is the adaptive response of cellular and extracellular compartments of the heart to mechanical or hormonal activation leading to changes in shape, volume, and mass of the left ventricle.<sup>3</sup> Key initiators of this remodeling process are ischemia, pressure overload, aging, and viral infection.<sup>4</sup> Nonstructural proteins are important modulators of ECM rearrangement in response to cardiac injury, demonstrated by their increased expression on cardiac injury.<sup>5</sup> Numerous functions of these proteins are generally ascribed to their different structural protein domains, yet when and why these protein domains have such diverse and sometimes even opposing functions are rarely addressed. A part of the explanation lies in proteolytic processing of nonstructural proteins by matrix metalloproteases (MMPs), which can release previously masked epitopes increasing functionality.<sup>6</sup> This well-accepted concept,

however, does not explain why, under pathophysiological conditions, these core protein domains are released at specific times by ubiquitously expressed enzymes. Whereas, glycosylation may expand the functional plasticity of these proteins by influencing protein folding, through protection from degradation or even by altering epitope recognition. Moreover, some sugars even have independent functionalities and hence are accountable for many important biological protein functions. We think that attention must be shifted toward the prominent biological process of glycosylation, which increases the functional range of ECM and allows for a more complete explanation of spatiotemporal, functional variability of core protein domains. For the purpose of this review, we would like to place glycobiology at the heart of our reassessment of cardiac ECM.

### Glycosylation: What Is It and Why Is It Important?

Glycosylation, the addition of sugars before protein secretion, is one of the most prominent and complex forms of post-translational modification in biology.<sup>7,8</sup> More than 50% of proteins in vertebrates are predicted to be glycosylated, allowing for enormous diversity in both structure and function.<sup>9</sup> Sugars were first demonstrated in eukaryotes in 1930 and ≈40 years later in bacteria and archaea.<sup>10</sup> However, they are also present in living fossils (cyanobacteria isolated from marine stromatolites), suggesting that eukaryotes may have inherited this glycosylation machinery from bacteria.<sup>8,11</sup> Accordingly, sugars are 1 of the 4 basic components of eukaryotic cells and are present intracellularly, extracellularly, or on the surface.<sup>10</sup> They modulate various biological processes such as cell adhesion, signal transduction, immunity, embryonic development, and microbial recognition, to name a few.<sup>12,13</sup> The most common forms of glycosylation in mammalian cells are N-linked and O-linked and can modify both intracellular and extracellular proteins and lipids. N-linked and O-linked glycosylation of nascent proteins takes place in the endoplasmic reticulum (ER) as well as the Golgi apparatus and entails many different enzymes.<sup>14,15</sup> The difference between N-linked and O-linked glycosylation lies in which amino acid they bind to, either the amide nitrogen N–H of an asparagine residue (N-linked) or hydroxyl –OH group of serine or threonine residues (O-linked). Via either N-linked or O-linked glycosylation, different sugar

### Cardiac Extracellular Matrix

GLYCOPROTEINS			GAGS	PROTEOGLYCANS			
Prototypical matricellular proteins	Fibers	Others		Hyaluronan	Hyalectans	Basement Membrane proteoglycans	Cell Surface proteoglycans
Thrombospondin	Collagens Elastins (not glycosylated)	Fibronectin Laminin		Versican	Perlecan Collagen XVIII Agrin	Syndecan Glypican	Class I Biglycan, Decorin, Asporin
SPARC			Class II Lumican, Fibromodulin, PRELP, Keratan, Osteoadherin				
Tenascin			Class III Osteoglycin, Epiphygan, Optican				
Osteopontin			Class IV Chondroadherin, Nyctalopin, Tsukushi				
Periostin			Class V Podocan, Podocan-like protein 1				
CCN							

**Figure 1. Classification of extracellular matrix (ECM) proteins.** The cardiac ECM is a complex meshwork of structural and non-structural proteins and sugars that are further subdivided into glycoproteins, proteoglycans, and glycosaminoglycans. Some proteins serve a structural (blue) function in the ECM (eg, collagens), whereas others have nonstructural (pink) roles, such as matricellular proteins. In addition, there are glycoproteins such as fibronectin that are able to do both (purple). This reservoir of glycosylated proteins allows for flexibility and diversity in how cardiac ECM functions in health and disease. GAG indicates glycosaminoglycan; and SPARC, secreted protein acidic and rich in cysteine.

chains such as glycans and glycosaminoglycans (GAGs) are bound to proteins. Branched oligosaccharides (glycans) alter protein function by influencing protein folding, stability, activity, distribution, targeting, and recognition<sup>16,17</sup> by forming glycoproteins. Unbranched linear polysaccharides consisting of repeating disaccharide units (GAGs) add biological function to proteins<sup>7,16,17</sup> by forming proteoglycans (Figure 2). Recapitulating, glycans are biologically active sugars that do not have independent functions, but merely alter original protein function, creating glycoproteins. GAGs are biologically active sugars with independent functions that can be found on their own or bound to a core protein in cardiac ECM, creating proteoglycans. GAGs thereby can both influence and enrich protein functionality. However, their biological function is not dependent on their attachment to a protein.

**GAGs: Great Potential for Sugars in Cardiac Matrix Biology**

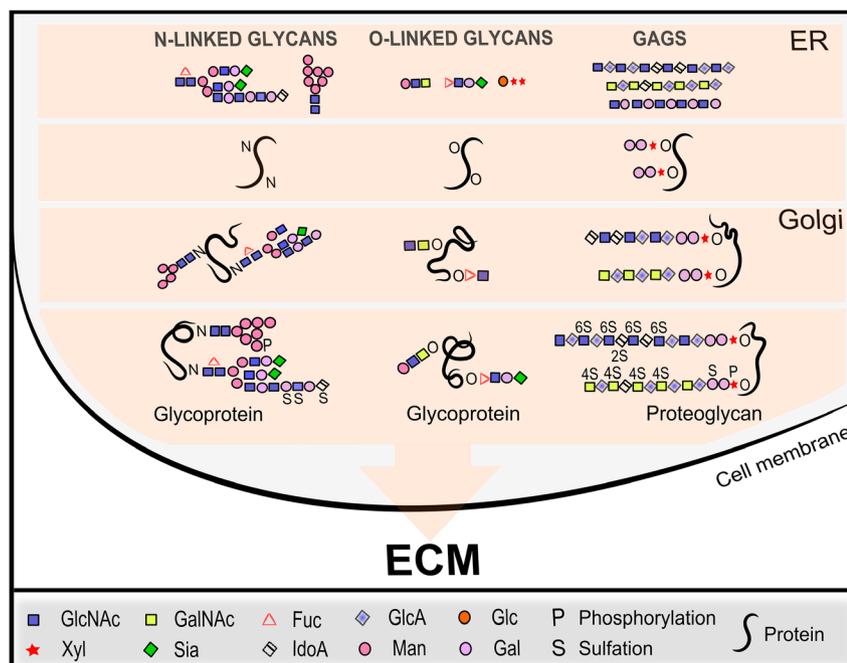
GAGs are among the most negatively charged molecules in mammalian tissues, allowing reversible and irreversible interaction with other matrix proteins, growth factors, and growth factor receptors in the ECM that bear positive charges on their surface. Their varied intracellular production and seemingly random attachment to proteins allow for wide-ranging protein functionality by altering or adding specific protein functions/characteristics. Before we consider the broad range of functions of most important bound GAGs in cardiac ECM, we will attempt to introduce some key biochemical concepts to facilitate the understanding of their functional variability.

**GAGs and Proteoglycans: Biosynthesis of Variable Polysaccharide Structures**

The broad range of functions of different GAGs can be attributed to their distinct assembly of sugars. Based on their core disaccharide structure, GAGs are subdivided into 4 major groups:

chondroitin sulfate (CS)/dermatan sulfate (DS), heparin/heparan sulfate (HS), keratan sulfate, and hyaluronan, also known as hyaluronic acid. All GAGs are produced in the ER and Golgi apparatus except for hyaluronan, which is not generated in the Golgi but formed at the plasma membrane. It, therefore, is not attached to a core protein and also not sulfated, in contrast to other GAGs.<sup>18,19</sup> To fully understand the importance of variation in GAG structures for GAG and proteoglycan function, we will briefly discuss O-linked GAG synthesis, exemplifying variability for all N-linked and O-linked GAGs and glycans. For more detailed information regarding N-linked or O-linked glycosylation, we would like to refer to specialized biochemical reviews.<sup>7,8</sup>

Translated proteins entering the ER are first modified by xylosyltransferases, adding xylose to a serine or threonine residue. Subsequently, in the Golgi apparatus, different enzymes are responsible for adding 2 galactose residues and a glucuronic acid, which completes the tetrasaccharide linker unit (Figure 2). The addition of fifth monosaccharide determines whether the sugar chain will become heparin/HS or CS/DS. Several enzymes such as EXT1 and EXT2 (glucuronyl/N-acetylglucosaminyl transferases) further elongate the heparin/HS chain by adding repeating disaccharide units after which epimerase converts glucuronic acid to iduronic acid. Epimerization is an important step in the formation of these GAG structures, because iduronic acid can change the important functional characteristic of the sugar chain. The elongation of CS/DS chain is being coordinated by 6 different chondroitin glycotransferases. Furthermore, epimerization of glucuronic acid to iduronic acid is required to form DS, although this is not a requisite for the formation of CS. Lastly, chains are modified by multiple N-, O-sulfotransferases and N-deacetylase,<sup>20</sup> which can further influence ultimate functional properties. The importance of all these specific enzymes in overall protein function is supported by a broad clinical manifestation of inherited glycosylation disorders in humans,



**Figure 2. Glycosylation.** Glycosylation of matrix proteins is a post-translational modification taking place in the endoplasmic reticulum (ER) and the Golgi apparatus of mammalian cells. Glycosylation is the addition of either glycans or glycosaminoglycans (GAGs) to the protein backbone. Glycans are bound via O-linked and N-linked glycosylation, whereas GAGs are mostly bound via O-linked glycosylation. Multiple enzymes enable the addition of these chains to the protein and are responsible for chain elongation and modification, such as sulfation (S) and phosphorylation (P), resulting in the formation of proteoglycans and glycoproteins. Depending on protein characteristics and enzymatic availability, any sugar chains can potentially be bound to any protein backbone, forming diverse proteoglycan or glycoprotein structures, as found in the extracellular matrix (ECM). Fuc indicates fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; GlcA, glucuronic acid; GlcNAc, N-acetylglucosamine; IdoA, iduronic acid; Man, mannose; Sia, sialic acid; and Xyl, xylose.

such as carbohydrate-deficient glycoprotein syndromes and congenital disorders of glycosylation.<sup>21</sup>

### GAGs and Proteoglycans: Customizing Sugars and Sugar-Protein Structures to Meet Environmental Needs?

Functional plasticity of GAG and proteoglycan structures is illustrated by the nontemplate-driven synthesis in the Golgi apparatus, which we briefly described in the previous section. Hence, what decides whether a sugar chain will become HS or DS and to what extent the chain is modified? Up to now, studies have mainly focused on investigating the role of total protein content in cardiac disease; as a result, the effect of proteoglycan/GAG composition and proteolytic processing on protein function is not often addressed. However, several important concepts have been established regarding GAG chain extension and modification. Beside important protein characteristics,<sup>22</sup> spatiotemporal distribution of the enzymes in ER and Golgi apparatus can influence the eventual GAG-protein structure produced by the cell.<sup>23,24</sup> In spite of extensive research, the sequential and mutual role of these 16 currently identified and differentially expressed enzymes, together with their colocalization in the Golgi apparatus, has not been completely clarified. Nevertheless, the significance of enzymatic chain modifications (sulfation) for ultimate functional characteristics of the GAG chain is well established, as illustrated by HS in HS proteoglycans syndecan and perlecan. HS is required for basic fibroblast growth factor (bFGF) receptor binding and activity, in both these HS proteoglycans.<sup>25</sup> Affinity-purified testing of several HS proteoglycan species, however, demonstrated high affinity of perlecan in promoting and activating bFGF receptor binding, whereas the affinity of syndecan-4 seems to be very low.<sup>26</sup> As it seems, bFGF binding by HS is dependent on differential structural requirements of the HS chain, such as the sulfation pattern, creating functional variability.<sup>25</sup> Still, whether there is a predetermined fixed GAG sequence remains elusive.<sup>27-29</sup> Yet, this vibrant glycosylation machinery in the Golgi enables the production of unfixed GAG or glycan structures, leading to unfixed proteoglycan or glycoprotein structures, thereby significantly increasing the biological variability of these proteins adjustable to environmental needs.

Beside increasing the functional variability by creating unfixed proteoglycan or glycoprotein structures, spatiotemporal proteolytic processing further increases the functional range of these single proteins. The significance of proteolytic processing in protein functionality is suggested by the membrane-bound HS proteoglycan syndecan-4. In the remodeling heart, full-length membrane-bound syndecan-4 and its shed extracellular ectodomain have very distinct effects.<sup>30</sup> Enzymatic shedding or degradation of syndecan-4 alters its structural conformation and thereby its function, again emphasizing protein plasticity by extracellular tailoring of GAG/proteoglycan structures in response to environmental needs.

### GAGs and Proteoglycans: Clinical Relevance of Studying Customized Sugars or Sugar-Protein Structures

The clinical relevance of glycosylation was first recognized in early 1930s by the discovery of blood groups, for which

different sugars are responsible.<sup>31</sup> Furthermore, heparin is one of the most commonly used antithrombotic drugs worldwide.<sup>32</sup> The importance of GAGs and proteoglycans in clinical practice is illustrated by the recall of heparin in 2008. In late 2007 and early 2008, the administration of anticoagulant heparin in hemodialysis patients caused serious adverse effects, including hypotension, swelling of the larynx and angioedema, and in some cases even death.<sup>33</sup> Oversulfated CS was later identified as a contaminant, explaining the anaphylactic response.<sup>33</sup> Further examination of heparin samples demonstrated the presence of N-acetyl glucosamine residues in a small heparin fraction.<sup>34</sup> Previous analysis already showed that the substitution of amino group of glucosamine residue with an acetyl group can subsequently change the function of heparin, confirmed by the clear nonanticoagulant properties of N-acetyl heparin.<sup>35,36</sup> In fact, the substitute groups on glucosamine residue determine its anticoagulant activity and in vivo deposition,<sup>35</sup> which illustrates the importance of understanding GAG biosynthesis and chain structure for clinical use.

### GAGs in the Heart: Challenging Candidates for Future Research

#### *Hyaluronan and Heparin*

Hyaluronan is the largest GAG in cardiac ECM and is the only one not attached to a core protein. It is known to form noncovalently linked complexes with proteoglycans. It was the first GAG discovered in 1934 by Karl Meyer and John W. Palmer<sup>37</sup> and, therefore, most comprehensively described in the heart.<sup>38-50</sup> The expression of hyaluronan is upregulated in experimental models of myocardial infarction (MI),<sup>50</sup> myocarditis,<sup>51</sup> and cardiac hypertrophy.<sup>43,49</sup>

In 1985, West et al<sup>52</sup> demonstrated that oligosaccharides derived from high-molecular-weight hyaluronan can have very distinct functions compared with the large hyaluronan variants. In intact ECM, hyaluronan exists mainly as a high-molecular-weight polysaccharide, whereas on injury, low-molecular-weight fragments are generated.<sup>53</sup> These hyaluronan fragments induce proinflammatory signaling in endothelial cells and leukocytes; their clearance through a CD44-dependent mechanism is a major step in the resolution of inflammatory response.<sup>54</sup> Moreover, hyaluronan/CD44 signaling plays an important role in promoting wound healing.<sup>55</sup> Hyaluronan is essential for transforming growth factor (TGF)- $\beta$ -induced myofibroblast differentiation.<sup>56-60</sup> CD44-null fibroblasts exhibit impaired responses to TGF- $\beta$ ,<sup>54</sup> highlighting the potential involvement of hyaluronan/CD44 signaling in cardiac repair. Lastly, an in vitro study suggests protective effects of hyaluronan on peroxide-treated cardiomyocytes.<sup>40</sup>

Implications in cardiac remodeling have also been demonstrated for heparin, a GAG secreted by mast cells. The beneficial effects of N-acetyl heparin administration have been demonstrated in rodent MI as suggested by preserved cardiac function and reduced infarct size.<sup>61-65</sup>

#### *HS, CS, and DS*

Published evidence suggests that HS, CS, and DS are important in cardiac development and in the pathogenesis of valvular heart disease.<sup>66-74</sup> Also, GAGs are important in

cardiac remodeling,<sup>75</sup> more specifically in cardiac hypertrophy,<sup>76</sup> age-related degeneration of the myocardium,<sup>77,78</sup> and MI.<sup>79,80</sup> Their importance is suggested by the increased expression of xylotransferase I, the enzyme responsible for attaching the GAG tetrasaccharide linker unit, and CS in patients with dilated cardiomyopathy.<sup>75</sup> This upregulation is also seen in fibroblasts stimulated with TGF- $\beta$  or subjected to mechanical stress.<sup>75</sup> Some experimental studies support a potential role for GAGs in cardiac remodeling after MI, demonstrated by the increase of CS and DS expression in myocardial scars of dogs and rats.<sup>80,81</sup> In fact, rats receiving gene transfer therapy with vascular endothelial growth factor immediately after MI show increased presence of CS and HS and a clear downregulation of heparanase (a HS-degrading enzyme),<sup>79</sup> which has been associated with improved cardiomyocyte survival and enhanced revascularization, recovery, and function.<sup>82</sup> The significance of GAGs in tissue organization and repair is further supported by the association of tissue degeneration with decreased GAG content in porcine aortic valves.<sup>78</sup> Remarkably, in aged rat myocardium, alterations occur in the sulfation pattern of HS side chains leading to structural and functional changes, such as the altered capacity of potentiating growth factor function.<sup>77</sup> Most importantly, heparin and HS can inhibit angiotensin II-induced hypertrophy in rat neonatal cardiomyocyte, indicating great therapeutic potential for these GAGs in hypertrophic cardiomyopathies.<sup>83</sup>

### Proteoglycans in the Heart

Matrix proteoglycans are divided into subgroups according to their extracellular localization, size, and structural properties (Figure 1); they include hyalectans (versican, aggrecan, neurocan, and brevican), basement membrane proteoglycans (perlecan, collagen type XVIII, and agrin), cell surface proteoglycans (syndecans and glypicans), and small leucine-rich proteoglycans (SLRPs, such as biglycan, decorin, lumican, and osteoglycin).<sup>84</sup> Although studies into the role of matrix proteoglycans in the heart are limited, we will focus on proteoglycans that have been implicated in cardiac pathology and will briefly discuss their effects in other systems that may be relevant to cardiac pathophysiologic conditions (Table).

#### Cell Surface Proteoglycans: Syndecans

Syndecans are a family of 4 transmembrane receptors consisting of a conserved extracellular ectodomain, a transmembrane domain, and a very short cytoplasmic domain.<sup>85</sup> The extracellular ectodomain is unique for every syndecan, yet it contains conserved GAG attachment sites, of which HS is most prevalent.<sup>86</sup> The extracellular domain of syndecan, with its HS/CS side chains, can modulate interactions with other matrix proteins, growth factors, or growth factor receptors.

Transcript levels of all 4 syndecan members increase in the myocardium of mice and rats on injury.<sup>85,87–89</sup> Increased syndecan-1 and syndecan-4 expression is of great significance in infarct healing,<sup>90</sup> protecting against cardiac dysfunction and dilatation.<sup>30,91</sup> Indeed, loss-of-function studies show an important role for syndecan-1 in proper infarct healing. Fourteen days after MI, mice lacking syndecan-1 demonstrate increased leukocyte recruitment and impaired formation of collagen fibers in infarct area.<sup>91</sup> In fact, overexpressing syndecan-1

further protects against cardiac dysfunction and dilatation by reducing inflammation and by improving collagen quality in the infarct<sup>91</sup>; these findings may suggest therapeutic potential. However, the effects of syndecan-1 in experimental models of cardiac remodeling are not always protective, reflecting the diverse functions of the molecule. Increased syndecan-1 expression during angiotensin II-induced pressure overload increases cardiac fibrosis by increasing CCN-2 and collagen expression, thereby inducing cardiac dysfunction.<sup>92,93</sup>

Evidence from loss-of-function studies suggests that syndecan-4 also plays an important role in cardiac remodeling after MI. Syndecan-4-null mice demonstrate increased cardiac rupture and worse cardiac function after MI by impaired granulation tissue formation.<sup>30</sup> Lack of syndecan-4 also impairs fibroblast function and bFGF-induced endothelial cell proliferation and tube formation.<sup>30</sup> Overexpressing syndecan-4 with an adenoviral vector in a rat MI model further supports the protective role of this proteoglycan, possibly mediated by inducing angiogenesis and by inhibiting inflammation and fibrosis.<sup>94</sup> Whereas, the overexpression of syndecan-4 ectodomain seems to be deleterious. As mentioned previously, overexpressing the extracellular domain of syndecan-4 impairs cardiac function and increases cardiac rupture after MI by impairing granulation tissue formation.<sup>30</sup> This syndecan-4 ectodomain produced by the adenoviral-overexpressing vector, similar to syndecan-4 produced by enzymatic shedding, acts as a dominant-negative inhibitor of endogenous syndecan-4.<sup>30</sup> Although lack of syndecan-4 is associated with impaired wound healing after MI mainly by influencing fibroblast/leukocyte recruitment and function, a cardiomyocyte-specific function may affect the outcome simultaneously. In a model of ischemia/reperfusion injury, syndecan-4-null mice exhibited increased myocardial damage because of enhanced cardiomyocyte apoptosis.<sup>95</sup> Hence, a lack of syndecan-4 is associated with increased infarct size. However, 7 days after ischemia/reperfusion, syndecan-4 loss is also associated with increased cardiomyocyte area and enhanced nuclear factor of activated T-cell activity in the border infarct and remote left ventricle, with accompanying improved cardiac function.<sup>95</sup> In fact, by inhibiting nuclear factor of activated T-cell signaling, syndecan-4-null mice develop left ventricular dilatation and dysfunction in response to aortic banding, instead of concentric hypertrophy as found in wild-type animals.<sup>96</sup> The effects of syndecan-4 on both cardiomyocytes and fibroblasts/leukocytes demonstrate different functions during different stages of the disease. This might be a consequence of differential glycosylation patterns demonstrating its flexibility as a cell surface proteoglycan; however, further research is needed to confirm this hypothesis.

#### Hyalectans: Versican

The dynamic ECM comprises various multifunctional proteins, which confer not only structural integrity but also the bioavailability of growth factors and cytokines to the surrounding tissue. Hyalectans, a group of proteoglycans named after their lectin-binding properties and their ability to bind hyaluronan,<sup>97</sup> are among these multifunctional ECM proteins. The size of core protein of these proteoglycans varies between 50 and 400 kDa and contains  $\approx$ 100 to 150 GAGs. Hence, glycosylation

**Table. Overview of Different Matrix Elements and How They Can Influence Different Cardiac Diseases**

Matrix Element	Model	Species	Effect	Mechanism	Reference
Heparin/N-acetylheparin	I/R	Dog	Reduced infarct size	Inhibition of complement activation	61
Hyaluronan	MI	Mouse	Resolution of inflammation	Interaction with CD44	54
Syndecan-1	MI	Mouse	↓Cardiac rupture and dysfunction	Reduced leukocyte recruitment	91
Syndecan-1	AngII	Mouse	↑Cardiac fibrosis	↑Phosphorylation of Smad2, CCN2	92–93
Syndecan-4	MI	Mouse	↓Cardiac rupture and dysfunction	↑TNF- $\alpha$ , MCP-1, MMP-9, bFGF	30
Syndecan-4	I/R	Mouse	↓Cardiomyocyte apoptosis	NFAT activity	95
Syndecan-4	AB	Mouse	↑Left ventricular hypertrophy	Activation of the calcineurin–NFAT pathway	96
Osteoglycin	AngII	Mouse	↑Left ventricular hypertrophy	ND	131
Biglycan	MI	Mouse	↓Cardiac rupture and dysfunction	Stabilizes collagen matrix, ↑TGF- $\beta$ and NO synthase	143–145
Decorin	SHR	Rat	↓Cardiac fibrosis and dysfunction	↓TGF- $\beta$ /p38 MAPK	152
Thrombospondin-1	MI	Mouse	↓Infarct expansion	↓MCP-1, IL-1, IL-6, TGF- $\beta$ , CXCL10	175
Thrombospondin-1	TAC	Mouse	↓Left ventricular hypertrophy and dilation	↑TGF- $\beta$ signaling, myofibroblast differentiation	177
Thrombospondin-1	Diabetes mellitus	Mouse	↓Cardiac dilation and promotes vascular rarefaction	↑Angiotensin-2	178
Thrombospondin-2	AngII	Mouse	↓Cardiac rupture	↓MMP-2 and -9 activity	176
Thrombospondin-2	VM	Mouse	↓Cardiac inflammation	↑Regulatory T-cell activity	182
Thrombospondin-2	Aging	Mouse	↓Mortality, cardiac dilation and fibrosis; ↑systolic function	Activation of prosurvival Akt signaling in cardiomyocytes and inhibition of MMPs	179
Thrombospondin-4	TAC	Mouse	↓Left ventricular hypertrophy	ND	186
Thrombospondin-4	Various	Mouse	↓Myocardial injury	Binds activating transcription factor 6 $\alpha$	187
Osteonectin	MI	Mouse	↓Cardiac rupture and dysfunction	↑Phosphorylation of Smad2	192
Osteonectin	TAC/aging	Mouse	Stabilizes collagen matrix	Postsynthetic procollagen processing	193–194
Tenascin-C	MI	Mouse	↑Cardiac fibrosis and dysfunction	ND	213
Osteopontin	MI	Mouse	Promoting collagen synthesis	ND	230
Periostin	MI/Ang	Mouse	↓Cardiac rupture and fibrosis	↑Fibroblast activity and collagen deposition	253,254
Periostin	MI	Swine	↑Cardiac function; ↓infarct size but ↑fibrosis	↑Cardiac myocyte proliferation	255
CCN-1	AIM	Mouse	↓Cardiac inflammation	↑Monocyte transmigration	272
CCN-2	I/R	Mouse	Reduced infarct size	↓GSK-3 $\beta$ activities, ↑phospho-SMAD2	262
CCN-2	Ang	Mouse	↑Cardiac fibrosis and dysfunction	↑TGF- $\beta$ -SMAD	268
CCN-5	Ang	Mouse	↓Cardiac fibrosis and dysfunction	↓TGF- $\beta$ -SMAD	268

AB indicates aortic banding; AIM, autoimmune myocarditis; Ang, angiotensin; bFGF, basic fibroblast growth factor; GSK, glycogen synthase kinase; IL, interleukin; I/R, ischemia/reperfusion; MAPK, mitogen-activated protein kinase; MCP, monocyte chemoattractant protein; MI, myocardial infarction; MMP, matrix metalloproteinase; ND, not defined; NFAT, nuclear factor of activated T-cell; SHR, spontaneous hypertensive rat; TAC, transaortic constriction; TGF, transforming growth factor; TNF, tumor necrosis factor; and VM, viral myocarditis.

further increases their molecular weight, as a result of which they are found in the ECM with sizes ranging from 1000 to 2500 kDa.<sup>98–100</sup> Further alternative splicing of the versican gene, which encodes the GAG chain-binding sites, generates  $\geq 4$  isoforms with different molecular weights, creating even more variation. These large matrix components interact with other matrix proteins, growth factor, and cell surface receptors.

Versican is ubiquitously expressed throughout the body and was first appreciated in the joints and cartilage for its hygroscopic properties. Attracting or holding water in the ECM is very important for enabling cell motility during development and disease. Studies regarding the functions of versican in the heart show that it is a much needed component in

cardiac ECM during cardiac development<sup>66,67,101,102</sup> and may be involved in the pathogenesis of valvular disease.<sup>102–104</sup> Unfortunately, not much is known regarding the specific functions of versican in conditions associated with cardiac remodeling, such as MI, angiotensin-induced cardiac hypertrophy, aging, or myocarditis. However, versican may exert important actions on the remodeling heart by modulating cytokine and growth factor responses and through interactions with other components of the ECM. Versican can bind many matrix components and inflammatory mediators, including hyaluronan, type I collagen, tenascin-R, fibulin-1 and fibulin-2, fibrillin-1, fibronectin, and chemokines.<sup>105</sup> Moreover, versican binds several cell surface receptors,

such as CD44,  $\beta$ 1 integrin epidermal growth factor receptor, L- and P-selectin,<sup>106</sup> low-density lipoprotein, glycoprotein ligand-1,<sup>105,107</sup> and toll-like receptor (TLR)-2,<sup>108</sup> and may modulate a wide range of cellular responses, such as cell proliferation, motility, and inflammatory activation. In vitro studies have suggested important actions of versican in modulating inflammatory responses; these actions could be important in a wide range of cardiac pathophysiologic conditions including MI and myocarditis. For instance, versican binding to hyaluronan may promote leukocyte adhesion to the ECM,<sup>106,109,110</sup> which may trigger CD44 signaling.<sup>106</sup> Also, versican-mediated stimulation of TLRs after injury elicits proinflammatory cytokine production and, therefore, enhances leukocyte attraction.<sup>111</sup> Recruited leukocytes comprising monocytes show a clear induction of versican expression, which is further induced on differentiation toward macrophages.<sup>112</sup> In the heart, versican induction is also observed in monocytes that infiltrate the myocardial infarct.<sup>113</sup> It has been even proposed that versican is related to macrophage polarization, a key cellular event in myocardial inflammation and repair, suggested by decreased versican gene expression in M2 versus M1 human macrophages.<sup>114</sup>

### Basement Membrane Proteoglycans: Perlecan

Perlecan is a large basement membrane proteoglycan most predominantly found in vascular ECM. It contains both pro- and antiangiogenic properties, which are partly mediated through the binding capacities of its HS with FGF-2.<sup>115,116</sup> The specific role of perlecan and its HS side chains in angiogenesis remains to be elucidated. It has been speculated that varied biochemical GAG and protein-GAG structures together with their proteolytic processing may be responsible for the broad and seemingly inconsistent functional range of perlecan. Our knowledge on the role of perlecan in the myocardium is limited to studies demonstrating its crucial role in cardiac development.<sup>117–119</sup> Whether perlecan is involved in the regulation of angiogenesis after myocardial ischemia or infarction remains unknown.

### Small Leucine-Rich Proteoglycans

SLRPs represent a group of extracellular proteins with similar low molecular weight (36–42 kDa) and structural organization comprising leucine-rich repeats flanked by cysteine residues. SLRPs are divided into 5 different protein classes (Figure 1) based on their unique composition of tandem leucine-rich repeats.<sup>120</sup> Each SLRP can be differentially glycosylated by the attachment of various types of glycans and GAGs. After original identification of these proteoglycans in the bone and cartilage,<sup>121</sup> their involvement has expanded to cancer biology, immunity, and embryonic development.<sup>122–124</sup> As a result of their diverse protein cores and GAG side chains, SLRPs interact with various cytokines, growth factors, cell surface receptors, as well as other matrix proteins. Indeed, they can bind different types of collagens,<sup>125</sup> TLRs,<sup>123</sup> epidermal growth factor receptors and insulin growth factor receptors,<sup>126</sup> low-density lipoprotein receptors,<sup>127</sup> and TGF- $\beta$ .<sup>128</sup> These interactions illustrate the involvement of SLRPs in a wide range of cellular functions and pathophysiologic responses, including collagen fibril assembly,<sup>125</sup> inflammation,<sup>123</sup> cell

proliferation,<sup>126</sup> atherosclerosis,<sup>127</sup> and fibrosis,<sup>128</sup> hence emphasizing their potential role in cardiac matrix biology.

The expression pattern of SLRPs supports their possible involvement in cardiac homeostasis and remodeling. Indeed, SLRPs decorin, biglycan, and lumican are ubiquitously expressed in mitral valves.<sup>70,129</sup> After mitral regurgitation, decorin expression increases in mitral valve leaflets,<sup>130</sup> whereas in the left ventricle, decorin and lumican expression decreases.<sup>131</sup> Remodeling of the left ventricle in response to mechanical or hormonal activation leads to changes in the expression of matrix components like these SLRPs. In patients with aortic stenosis, the expression of SLRP osteoglycin is markedly increased and shows a strong correlation with left ventricular mass, illustrating the association between SLRPs and cardiac remodeling.<sup>132</sup> Although all members of SLRPs are important regulatory ECM components,<sup>132,133</sup> we will only focus on the main members that have been implicated in myocardial pathology. However, published evidence on the specific functions of SLRPs in cardiac remodeling is limited.

### Class I SLRPs: Biglycan and Decorin

Biglycan and decorin are members of class I SLRPs and contain either CS or DS side chains, which are often highly sulfated. These SLRPs modulate ECM organization, cellular adhesion, and migration.<sup>133</sup> Their expression is ubiquitous in the normal heart<sup>134–136</sup> and increases in response to pressure overload<sup>137–139</sup> and MI.<sup>140–142</sup> Biglycan seems crucial in infarct healing by ensuring proper collagen scar formation through stimulating collagen fibril assembly and thereby prevents infarct dilatation and overall dysfunction.<sup>143,144</sup> Transgenic mice overexpressing human biglycan upregulate the expression of proteins such as TGF- $\beta$  and the NO synthase family, suggesting a potential role for biglycan in cardiac remodeling and cardioprotection.<sup>145</sup> In vitro studies suggest a cytoprotective effect for biglycan in neonatal rat cardiomyocytes mediated through the upregulation of endothelial NO synthase transcript and protein levels and a subsequent increase in cardiomyocyte NO content.<sup>146</sup> Biglycan may modulate the inflammatory response by binding with TLR-2 and TLR-4<sup>147–150</sup> and may be involved in fibrosis by binding to collagen fibrils.<sup>125</sup>

Myocardial decorin expression increases after infarction and correlates with collagen deposition, TGF- $\beta$  levels, and SMAD expression.<sup>140,142,151</sup> Decorin may negatively regulate fibrosis. In vitro addition of exogenous decorin significantly decreased collagen production by TGF- $\beta$ -stimulated human cardiac fibroblasts.<sup>152</sup> In vivo, decorin overexpression with an adeno-associated viral vector post-MI leads to reduced cardiac fibrosis and improves cardiac function.<sup>153</sup> Decorin may be implicated in the pathogenesis of cardiac fibrosis through binding to TGF- $\beta$ <sup>128</sup> and by associating with collagens I and III,<sup>154,155</sup> thus playing an important role in collagen cross-linking. Decorin DS side chain is involved in proper collagen assembly and mediates cellular adhesion to the ECM.<sup>156</sup> Biological relevance of this GAG in matrix structure is illustrated by thinner collagen fibers and increased focal adhesion in mice lacking decorin. In contrast, mice lacking only the DS side chain have thicker collagen fibrils.<sup>156</sup> Much like biglycan, decorin can act as an endogenous ligand for TLR-2 and TLR-4 stimulating the production of proinflammatory cytokines in

macrophages<sup>157</sup> and may play a role in the activation of myocardial inflammatory responses.

### **Class II SLRPs: Lumican**

Lumican is ubiquitously expressed in adult mice where the heart and the eye showed the highest levels of expression.<sup>158</sup> The modification of lumican with keratan sulfate to form a proteoglycan is crucial for corneal transparency.<sup>159</sup> The attachment of keratan sulfate to lumican mainly occurs in the eye, whereas in other organs such as the heart, lumican is present as a glycoprotein without any GAGs attached. However, increased lumican expression in rat heart after ischemia/reperfusion consists of both the proteoglycan and the glycoprotein, which are differentially expressed during different phases of wound healing.<sup>160</sup> Indeed, alterations in glycosylation of decorin and lumican core protein in response to pressure overload might be important in cardiac remodeling, with a potential important role for CS/DS GAG chain-synthesizing enzymes.<sup>161</sup> Interestingly, both lumican and decorin can be cleaved by MMP-1, suggesting that proteolytic processing is very precisely regulated and crucial for proteoglycan-mediated spatiotemporal functions.<sup>162</sup>

Like biglycan and decorin, lumican is also important in modulating the inflammatory response by presenting lipopolysaccharide to CD14, thereby activating TLR-4 in macrophages.<sup>163</sup> Furthermore, lumican regulates neutrophil infiltration in a murine keratitis model, by interacting with CXCL1, thus creating a chemokine gradient along which these neutrophils infiltrate the injured tissue.<sup>164</sup> By interacting with collagens and other matrix components such as aggrecan and integrins,<sup>125</sup> lumican modulates matrix rearrangement and collagen assembly, as supported by decreased hepatic fibrosis, impaired collagen fibrillogenesis, and increased matrix turnover on liver injury in mice lacking lumican.<sup>165</sup> The diversity of this SLRP and its potential to interact with many matrix components, cytokines, and growth factors, together with the upregulation of lumican in experimental and clinical heart failure,<sup>166–168</sup> make it a very interesting candidate for future research.

## **Glycoproteins in the Heart: Matricellular Proteins**

As described above, a part of the nonstructural role of the ECM is assigned to a family of structurally unrelated glycoproteins, which have been termed matricellular proteins by Paul Bornstein.<sup>169,170</sup> The first proteins classified in this group were secreted protein acidic and rich in cysteine (SPARC), thrombospondin (TSP)-1, and tenascin-C; osteopontin (OPN), periostin, TSP-2, TSP-4, tenascin-X, CCN-1 (cysteine-rich protein-61), and CCN-2 (connective tissue growth factor) were later added to this group of prototypical matricellular proteins. For the purpose of this review, we will focus on select matricellular proteins with a key role in cardiac remodeling (see the Table). A comprehensive review can be found elsewhere.<sup>1</sup>

### **Thrombospondins**

This family of large secreted glycoproteins, consisting of 5 members, is subdivided into 2 different groups based on their structural organization and oligomerization status. Members of the first group, TSP-1 and TSP-2, form trimers, whereas

the remaining members, TSP-3 to -5, arrange as pentamers.<sup>171</sup> Up to now, research has focused more on the extensive role of trimeric TSPs in the heart, though some recent studies have unveiled the involvement of pentameric TSPs, in particular TSP-4, in cardiac disease. As typical matricellular proteins, TSPs are not present in normal adult ECM, but their expression increases greatly in response to injury and during cardiac development. TSP-1 is transiently expressed during cardiac development, whereas TSP-2 expression is abundant in connective tissues of many organs.<sup>172</sup> TSP-3 to -5 expression during development is restricted to specific organs, such as the brain, cartilage, lung, and nervous system.<sup>172–174</sup>

TSPs are of key importance in cardiac pathology. TSP-1 and TSP-2 are protective after MI and pressure overload by preserving the cardiac matrix possibly via inhibition of MMP activity and by facilitating TGF- $\beta$  activation (specific for TSP-1).<sup>175–177</sup> TSP-1 is a potent angiostatic mediator; in diabetic heart, TSP-1 has been shown to promote vascular rarefaction by enhancing angiopoietin-2 expression.<sup>178</sup> In aging heart, TSP-2 has protective effects by activating prosurvival Akt signaling and by inhibiting MMP activity.<sup>179</sup>

Furthermore, the functional domains of TSP-1 and TSP-2 can interact with collagens and with a variety of other matrix components, such as cytokines, growth factors, and proteases, altering their activity.<sup>180</sup> Also, TSP-1 and TSP-2 are capable of changing focal (strong) adhesion to a more intermediate state as suggested by its typical deadhesive properties, similar to those of other matricellular proteins such as SPARC and tenascin-C.<sup>181</sup> Lastly, TSP-1 and TSP-2 demonstrate anti-inflammatory properties,<sup>182,183</sup> mainly by increasing regulatory T-cell activation.<sup>182,184</sup> The increased number of regulatory T-cells was shown in murine hearts of wild-type mice with viral myocarditis compared with their knockout littermates.<sup>182</sup> More specifically, a CD47-specific TSP peptide containing the C-terminal domain promotes the generation of human regulatory T-cells that suppress proliferation and cytokine production of autologous T-cells.<sup>184</sup> Other effects of TSPs on modulating the immune response are mostly prompted by TSP-1/CD36 interaction, which regulates the clearance of apoptotic neutrophils<sup>185</sup> and TSP-1-initiated TGF- $\beta$  activation.

TSP-4 displays similar protective effects as TSP-1 and TSP-2 in a murine transaortic constriction model, suggested by increased heart weight and accentuated reactive fibrosis observed in TSP-4-null mice.<sup>186</sup> Interestingly, Lynch et al<sup>187</sup> recently suggested that the protective effects of TSP-4 in remodeling myocardium may be because of the augmentation of cardiomyocyte ER function through the effects on nuclear shuttling of activating transcription factor 6 $\alpha$ , leading to reduced protein synthesis, enhanced degradation of damaged or misfolded proteins, and selectively induced expression of protective proteins. The upregulation of TSPs in human patients with aortic stenosis (TSP-2)<sup>176</sup> or end-stage dilated cardiomyopathy (TSP-4)<sup>188</sup> further supports the importance of TSPs during cardiac remodeling. The involvement of TSPs in human cardiac disease was supported by findings demonstrating an association between single nucleotide polymorphisms in TSPs and premature coronary atherothrombotic disease.<sup>189</sup>

### Secreted Protein Acidic and Rich in Cysteine

The classical matricellular protein SPARC, also known as osteonectin because of its original detection in the bone,<sup>190</sup> consists of a EF-hand calcium-binding domain, a follistatin-like domain, and a kazal serine protease inhibitor domain.<sup>191</sup> SPARC has high affinity for collagen and facilitates collagen crosslinking. The latter is needed for proper infarct healing, because the absence of SPARC in mice caused increased cardiac rupture and dysfunction after MI.<sup>192</sup> In contrast, this increased collagen crosslinking is detrimental in pressure overload<sup>193</sup> and aging,<sup>194</sup> because it induces increased diastolic dysfunction. Hence, the expression of SPARC after MI<sup>195</sup> is beneficial, whereas the expression in response to pressure overload<sup>193</sup> and as a consequence of aging<sup>194</sup> can be detrimental for maintaining cardiac function.

Proteolytic cleavage of SPARC by several MMPs (including MMP-2, -3, -7, and -13) modulates its function, thereby increasing the affinity for collagen<sup>196,197</sup> or releasing smaller proteolytic protein fragments that can, for instance, modulate angiogenesis.<sup>198</sup> As a classical matricellular protein, SPARC features deadhesion properties<sup>181</sup> and modulates the activity of various growth factors crucially involved in tissue repair, angiogenesis, and fibrosis, such as FGF-2,<sup>199</sup> vascular endothelial growth factor,<sup>200</sup> platelet-derived growth factor,<sup>201</sup> insulin-like growth factor-I,<sup>202</sup> and TGF- $\beta$ .<sup>203,204</sup>

### Tenascins

Tenascins are a group of proteins consisting of 4 highly conserved hexameric glycoproteins termed tenascin-C, -X, -R, and -W. Two members of the tenascin family (tenascin-C and -X) modulate cell migration, adhesion, and growth in a typical matricellular manner.<sup>205–207</sup> Several common functional domains are present in this group, such as epidermal growth factor–like repeats, fibronectin type III domains, and a fibrinogen globe.<sup>208</sup> Tenascin-C is markedly expressed during embryonic development, mainly in connective tissues; however, its expression is suppressed in adult tissues and reappears on injury or remodeling and in response to mechanical strain in fibroblasts.<sup>209</sup> Various factors released during cardiac remodeling, such as FGF-2 and TGF- $\beta$ , increase tenascin-C expression, which suggests that it may be important for regulating fibrosis and inflammation. Tenascin-C is strongly induced in inflammatory processes and may regulate leukocyte recruitment in a context-dependent manner.<sup>210,211</sup> Moreover, it can activate TLR-4 as an endogenous ligand.<sup>212</sup> Tenascin-C loss has protective effects in postinfarction remodeling<sup>213</sup>; however, the mechanisms responsible for these actions remain poorly understood. Like all matricellular proteins, tenascin-C can induce a deadhesive state in fibroblasts, potentially by prohibiting integrin-mediated attachment by binding fibronectin.<sup>214,215</sup> In the heart, upregulation of tenascin-C after MI,<sup>216</sup> pressure overload,<sup>217</sup> or myocarditis<sup>218</sup> is a characteristic of cardiac remodeling. Consequently, when this upregulated glycoprotein is released into the blood stream of human patients with a wide range of cardiac conditions, it becomes a reliable biomarker predicting the degree of cardiac remodeling and subsequent mortality.<sup>219–222</sup>

### Osteopontin

First identified in the bone in 1985,<sup>223</sup> OPN, also known as bone sialoprotein I or early T-lymphocyte activation, has all

the characteristics of a matricellular protein and is highly upregulated on injury.<sup>224</sup> OPN consists of a calcium-binding domain and larger integrin-binding domains, which is cleaved by thrombin,<sup>225</sup> releasing an integrin-binding site that is involved in leukocyte adherence.<sup>226</sup> Experimental models of cardiac fibrosis and hypertrophy resulted in marked OPN upregulation,<sup>227,228</sup> which results in prohypertrophic<sup>229</sup> and profibrotic<sup>230</sup> responses. Furthermore, OPN expression increases on MI,<sup>231</sup> aging,<sup>232</sup> and valvular disease.<sup>233</sup> This secreted glycoprotein functions as a classical nonstructural matricellular protein but also has apparent cytokine-like properties.<sup>234,235</sup> OPN can interact with integrin receptors, including the vitronectin receptor and CD44, and hence is involved in bone mineralization, cancer biology, inflammation, wound healing, leukocyte function and recruitment, and cell survival.<sup>234</sup> Specifically in the heart, OPN upregulation in response to MI, primarily localized in macrophages,<sup>236</sup> is crucial for proper collagen deposition and reduction of chamber dilatation, thereby protecting from adverse cardiac remodeling.<sup>231</sup> Most importantly, in patients with heart failure,<sup>237</sup> MI,<sup>238</sup> or stenotic valvular<sup>239–241</sup> disease, OPN plasma levels seem to be a very promising biomarker for prediction<sup>242</sup> or progression<sup>243</sup> of heart diseases related to adverse cardiac remodeling.

### Periostin

This recently discovered glycoprotein was originally called osteoblast-specific factor 2 because of its bone function, and later renamed because of its high abundance in periosteum and periodontal ligament to periostin.<sup>244</sup> Periostin shares structural homology with axon guidance protein fasciclin-1, containing similar sequences that allow binding to integrins and GAGs.<sup>245</sup> Therefore, because of its high expression on injury<sup>246–248</sup> and potential to interact with other matrix components and integrins, periostin became accepted as a matricellular protein. Periostin is consistently elevated in response to myocardial injury<sup>249–252</sup> and associated with fibrosis. Its upregulation prevents cardiac rupture by stimulating fibroblast recruitment, myofibroblast transdifferentiation, and collagen deposition.<sup>253,254</sup> Indeed, experimentally controlled delivery of recombinant periostin peptide into the pericardial space in swine after MI results in increased fibrosis in the remote region 1 and 12 weeks after treatment.<sup>255</sup> In response to pressure overload, periostin also enables fibroblast recruitment, myofibroblast transdifferentiation, and collagen deposition, yet also induces slight cardiomyocyte hypertrophy without leading to decompensation.<sup>253</sup> Interestingly, periostin re-expression in mice after cardiac injury is selective for pathological stimuli because it is not re-expressed in response to physiological stimuli such as hypertrophy-induced forced swimming or voluntary wheel running exercise.<sup>253</sup> Although the levels of periostin in human patients with heart failure<sup>251</sup> or acute MI<sup>250</sup> are elevated, research regarding its diagnostic or therapeutic potential in humans is still warranted.

### CCN Family

The CCN family consists of 6 members and owes its name to its first members: cysteine-rich protein 61, connective tissue growth factor, and nephroblastoma-overexpressed protein, also known as CCN-1, -2 and -3. They consist of an insulin-like growth factor–binding domain, a von Willebrand factor type C repeat, and a

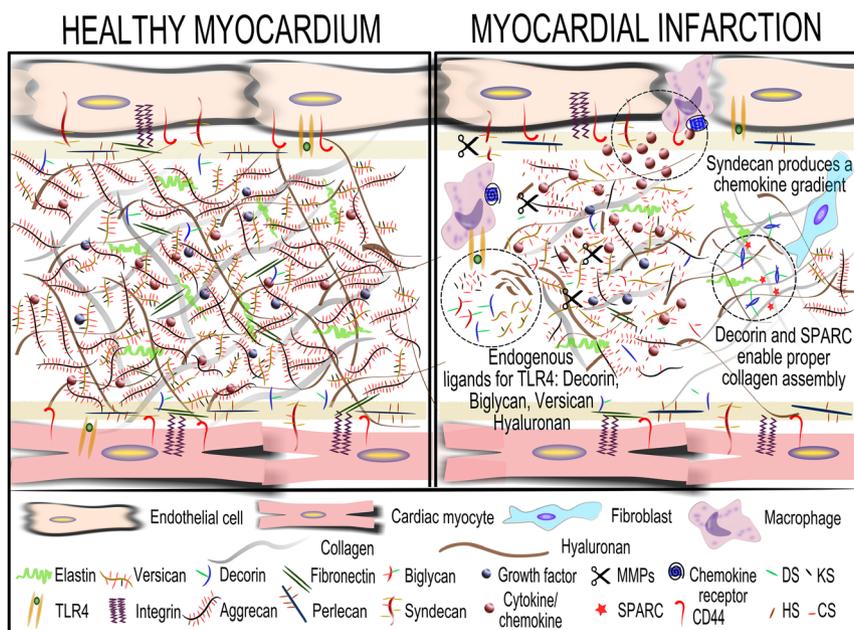
single TSR domain.<sup>256</sup> Although originally considered as growth factors, many studies over the past decades have found these to act as modulators of cell–ECM adhesion through their interactions with integrins, HS proteoglycans, growth factors, and cytokines.<sup>257</sup> Because of their identification as matricellular proteins, CCNs have been studied extensively in heart disease. Almost all CCN members show marked upregulation in response to injury. CCN-2 is the most comprehensively studied member of the CCN family in relation to cardiac disease. The evident upregulation of CCN-2 in response to MI<sup>258</sup> and pressure overload<sup>259–261</sup> is essential for potentiating TGF- $\beta$  signaling, stimulating survival of cardiomyocytes, and enabling proper angiogenic and fibrotic responses, both in MI<sup>262–264</sup> and in response to pressure overload.<sup>265–269</sup> In contrast, CCN-5 overexpression diminishes the hypertrophic and fibrotic response after pressure overload by influencing TGF- $\beta$ / SMAD3 signaling.<sup>268</sup> Furthermore, increased expression of CCN-1 in response to MI or pressure overload<sup>270</sup> may modulate phenotype and function of leukocytes, cardiomyocytes, and fibroblasts.<sup>271</sup> The overexpression of CCN-1 in experimental autoimmune myocarditis confirms the potential role of CCN-1 on leukocyte behavior by decreasing cardiac inflammation.<sup>272,273</sup> Finally, although the *in vivo* effects of CCN-4 upregulation after MI remain unclear, *in vitro* studies suggest a role for CCN-4 in fibroblast proliferation and transduction of prohypertrophic and prosurvival signals in cardiomyocytes.<sup>274–276</sup>

### Conclusions and Future Perspectives

The complex architectural arrangement of cardiac ECM is crucial for maintaining proper cardiac function and hence

requires tight regulation. Research in the past decades has focused on identifying the various proteins and proteases that serve as orchestrators of ECM rearrangement in response to injury. This is illustrated by the critical role of proteoglycans, glycoproteins, and GAGs in orchestrating cardiac ECM reorganization during tissue remodeling and in regulation of cardiac inflammation, angiogenesis, and fibrosis (Figure 3). In line with the search for new clinically relevant GAGs, proteoglycans, or glycoproteins in the cardiovascular field, we propose focusing on some promising candidates, such as perlecan, versican, hyaluronan, and SLRPs such as lumican, decorin, and biglycan, but also the less known SLRPs such as asporin, osteoglycin, and epiphycan. Understanding their biochemical structure in relation to their biological function may lead to the development of new biochemical structures or compounds with great therapeutic potential for patients with heart disease.

Up to now, the remarkable variety in functions of many glycoproteins, as well as of the less well studied proteoglycans, has been primarily attributed to their assortment of active protein domains, which can be shaped by process-specific proteases to increase their functional range. We think that sugars such as glycans and GAGs are crucial in directing, changing, and impeding proteolytic cleavage of proteins, customizing them to environmental needs, and as a result increasing their domain-based functions. Glycosylation is a much more comprehensive explanation as to how glycoproteins and proteoglycans broaden their functional range and contribute to the plasticity of cardiac ECM. However, research regarding the role of sugars,



**Figure 3. Complex cardiac extracellular matrix (ECM) with potential functions of some residing matrix elements.** In healthy myocardium, the ECM forms a complex architectural meshwork and cytokine reservoir, through the close interaction of all matrix components. These include hyaluronan, CD44, collagens, hyalactans, basement membrane proteoglycans, fibronectin, and integrins. On cardiac damage, macrophages invade the cardiac tissue via interactions with ECM components hyaluronan and fibronectin, in turn secreting proteases that degrade the ECM and releasing cytokines bound in the matrix. The degraded ECM elements may further amplify the immune response by either stimulating important danger signal receptors such as TLR-4 (eg, hyaluronan, decorin, and versican) or producing chemokine gradients for the recruitment of leukocytes (eg, syndecan). Furthermore, proteoglycans, such as decorin and biglycan, and glycoproteins, such as SPARC, enable proper collagen assembly. An independent role of glycosaminoglycans (CS, DS, and HS) is starting to emerge, further elaborating the extensive repertoire of the ECM in health and disease. CS indicates chondroitin sulfate; DS, dermatan sulfate; HS, heparan sulfate; KS, keratan sulfate; and TLR-4, toll-like receptor-4.

either alone or attached to a protein backbone, and their spatio-temporal function is still in the early phase, with a focus on the role of total GAG or proteoglycan content in the heart. It is the biochemically variable nontemplate-driven sugars, together with their position on the protein, that tremendously enhance potential interactions with other matrix proteins, growth factors, and growth factor receptors. Yet, there are challenges to be faced. GAGs are also subjected to specific degrading enzymes changing their appearance and function.<sup>277–279</sup> Hence, looking at the total GAG or proteoglycan content introduces difficulties in attributing functionality. So, how do we address the plasticity of these proteins and sugars in the ever-changing cardiac ECM? When investigating the role of specific proteoglycans, glycoproteins, or GAGs in transgenic animals, the type and range of glycosylation of the studied protein could be identified. The extent of glycosylation could be determined by using specific polysaccharide lyases<sup>280</sup> or preferably mass spectrometry.<sup>281</sup> Care must be taken when linking in vivo studies with in vitro experiments, because the glycosylation pattern can differ significantly. Furthermore, protein epitopes or sugars to which newly identified functions can be ascribed should be identified. A further obstacle is in understanding the plasticity of protein over time in animal disease models. In reality, many different cell types produce undefined GAG/glycans structures, hence undefined glycoprotein and proteoglycan structures, together with proteolytic processing, can influence protein appearance and function over time. Therefore, specific attention is needed in understanding what drives this tailor-made cell-specific glycosylation. How is it directed? Where can we intervene? Cell-specific and inducible transgenic animal models studying the role of specific enzymes in the ER and Golgi could give more insight in the sequential and mutual role of the 16 currently identified and differentially expressed enzymes, and hence explain a crucial determinant of ECM plasticity in response to injury. Understanding why and when these seemingly random modifications occur may lead to the discovery of very specific glycosylation profiles allowing well-tailored disease interventions.

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### Disclosures

None.

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