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Thrombospondins in the Transition from Myocardial Infarction to Heart Failure

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Abstract

The heart's reaction to ischemic injury from a myocardial infarction involves complicated cross-talk between the extra-cellular matrix (ECM) and many different cell types within the myocardium. The ECM functions not only as a scaffold where myocytes beat synchronously, but an active signaling environment that regulates the important post-MI responses. The thrombospondins are matricellular proteins that modulate cell - ECM interactions, functioning as "sensors" that mediate outside-in and inside-out signaling. Thrombospondins are highly expressed during embryonic stages, and although their levels decrease during adult life, can be re-expressed in high quantities in response to cardiac stress including myocardial infarction and heart failure. Like a swiss-army knife, the thrombospondins possess many tools: numerous binding domains that allow them to interact with other elements of the ECM, cell surface receptors, and signaling molecules. It is through these that the thrombospondins function. In the present review, we provide basic as well as clinical evidence linking the thrombospondin proteins with the post myocardial infarction response, including inflammation, fibrotic matrix remodeling, angiogenesis, as well as myocyte hypertrophy, apoptosis, and contractile dysfunction in heart failure. We will describe what is known regarding the intracellular signaling pathways that are involved with these responses, paving the road for future studies identifying these proteins as novel therapeutic targets for cardiac disease.

Keywords

thrombospondin; myocardial infarction; remodeling; angiogenesis; heart failure; TGF β

Introduction

Thanks to the efforts of the field, mortality from myocardial infarction (MI) has been steadily decreasing over the last several decades [1]. While this is undoubtedly a positive development, it has led to a population of patients who have survived an MI and are at higher risk of heart failure. The ischemic injury induces necrotic cell death, and the post-MI

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response involves apoptosis, inflammation, extracellular matrix remodeling, fibrotic scar formation, proliferation/transdifferentiation of myofibroblasts, angiogenesis, and scar maturation. These responses, while intended to limit the injury and help recovery in the short term, establish an environment that is now ripe for the progression to heart failure (HF). Thus, some time after their MI event, patients begin experiencing further remodeling, hypertrophy, dilation, and reduced systolic function.

For those who suffer an MI between the ages of 45 to 64, roughly 8% of men and 18% of women will develop HF within 5 years [2], and for those 65 years old or greater, that number increases to 20% and 23%, respectively. Considering that every 43 seconds one American will have an MI [2], these numbers can represent a major impact on health care. Furthermore, since the 5-year mortality from HF is 50%, preventing the transition from MI to HF is tremendously important. In order to accomplish this goal, we must first understand the mechanisms of the progression of the disease. It is in this environment that the thrombospondin family of matricellular proteins has been found to be quite promising.

The term “matricellular protein” was coined by Bornstein in 1995 [3] for extracellular matrix (ECM) proteins that do not contribute to the structural integrity of the ECM, but could modulate cellular function. There are a number of matricellular proteins including, osteopontin, periostin, secreted protein acidic and rich in cysteine (SPARC) [4], and the thrombospondin and tenascin families, almost all of which have important roles in cardiac disease and remodeling [5]. The thrombospondin (TSP) family of secreted calcium-binding glycoproteins consists of five members divided into two groups. TSP-1 and TSP-2 form homotrimers (Subgroup A), while TSP-3, TSP-4 and TSP-5 (COMP) form homopentamers (Subgroup B)[6, 7]. Structurally, the thrombospondins contain a carboxy-terminal domain similar to L-type lectin, followed by calcium-binding type 3 repeats, and several EGF-like domains, while the N-terminal domains tend to vary between the family members [8] (Figure 1).

The thrombospondins play an important role during development, as they are all highly expressed during this phase; however, mRNA and protein levels quickly drop to very low, almost undetectable levels in adults [9]. In response to stress, however, many of the thrombospondin proteins are overexpressed. Their ability to modulate function arises through their binding with elements of the ECM such as cell surface receptors and extracellular factors such as cytokines and growth factors (Table 1). This review will examine the roles the thrombospondin proteins play in the various stages of the heart's adaptive response to myocardial infarction and the eventual maladaptive changes that result in heart failure.

From Myocardial Infarction to Heart Failure

Myocardial infarction (MI) kills living heart muscle, leading to its replacement by scar. The acute insult produces early inflammation, activation of reactive oxygen species, and triggering of different molecular pathways that contribute to the loss of myocardium. This results in a rapid increase in loading conditions with a subsequent characteristic pattern of

remodeling that affects peri-infarct myocardium as well as the distant non-infarcted cardiac tissue (remote zone).

Left ventricular remodeling is a process by which different genetic, neurohormonal, and mechanical factors affect ventricular mass, shape, and size, with a consequent impact on function. A combination of pressure and volume overload, which the heart is now exposed to, prompt myocytes as well as non-myocyte cardiac cells to activate a signaling cascade that will change the architecture of the heart according to the new mechanical scenario, ultimately ending with an established scar, chamber hypertrophy, dilatation and fibrosis. The normal myocardium consists of 4 components that are highly interrelated: cardiac myocytes, cardiac fibroblasts, the microcirculation and the extracellular matrix. All four players are crucial in the development of chamber remodeling and hypertrophy. Left ventricular hypertrophy (LVH) is characterized by an increase in myocardial mass. Pathologic hypertrophy, for the purpose of this review, will be mainly focused on that occurring in the setting of hypertension (pressure overload) or after MI (pressure and volume overload). Matricellular proteins play an active role in post-MI cardiac remodeling, and specifically the thrombospondins have been shown to modulate all four components mentioned above.

It is difficult to parse out the independent effects of the TSPs on the different stages of progression from MI to HF, because these steps build upon each other. The inflammatory stage recruits the necessary components for remodeling and angiogenesis [10], and both of these determine the environment for contractile dysfunction and dilation. These stages overlap and have common contributors such as transforming growth factor β (TGF β), making it difficult to study an individual step even if extremely precise *in vivo* temporal genetic control was possible. This should be kept in mind as the present review attempts to discuss each stage independently.

Inflammation

With abundant cell death after the ischemic injury, the released cellular content and other damage signals drive a robust inflammatory response in the heart [11]. The damaged matrix, fibroblasts, and mast cells release chemokines and cytokines that recruit neutrophils first. Subsequently, monocytes take over, which differentiate into macrophages, based on specific environmental cues, which also help to remove the inflammatory material. Macrophages and regulatory T-cells also help to suppress the inflammatory reaction, and degrade the neutrophils. The inflammatory response post-MI is quite complex, and is described in greater detail in other reviews [12]. The inflammatory response is essential, as it clears out dead cells and other remnants and activates repair signals. However, a prolonged inflammatory response leads to adverse remodeling and poor outcomes from cardiac rupture [13, 14].

In addition to containing the inflammatory response temporally, it is necessary to contain it spatially so it does not expand beyond the infarcted zone and to living myocytes. There is a rapid and significant increase in TSP-1 expression post-MI [15] that is specifically localized to the border zone in both mouse and dog models of MI [16]. In TSP-1 knock-out mice, an invasion of the inflammatory response was seen into the non-infarcted region, including

expression of macrophages, myofibroblasts, and granulated tissue. This spatially unencumbered inflammatory response led to adverse ventricular remodeling (increase in LV size) [16]. The mechanism for this action is primarily through TSP-1's interaction with anti-inflammatory TGF β (Figure 2).

TGF β is an impressively pleiotropic signaling peptide that is a central regulator of post-infarct inflammation [17] as well as many other post-MI responses, including the fibrotic and proliferative components. Through its cell surface receptors (TBR1) it activates Smad, extracellular signal-regulated kinase (ERK), c-Jun-N-terminal kinase (JNK), p38 mitogen-activated kinase (MAPK), and other pathways [17]. When TGF β is secreted, it contains an N-terminal Latency Associated Peptide (LAP), which non-covalently associates with the active domain, conferring latency (inactivation). TSP-1 activates TGF β by binding to the LAP and inhibiting its interaction with the bioactive domain [18]. *In vivo*, TSP-1 is a major activator of TGF β [19, 20] and inhibits inflammation in this manner [21, 22].

TSP-1 can also inhibit inflammation through interaction with CD36. Acting as a molecular bridge, TSP-1 binds cell-surface CD36 on macrophages to neutrophils, which furthers apoptosis of the inflammatory neutrophils [23]. Other models of inflammation show that TSPs can also work through T-cells. Wild type (WT) mice could reduce oxazolone-induced inflammation within 2 days, while it took twice as long in mice deficient in TSP-1 or TSP-2 [24]. In the setting of acute viral myocarditis, TSP-2 KO mice experienced increased mortality and inflammation, with a decrease in activation of anti-inflammatory T-regulatory cells [25]. TSP can interact with CD47 to activate T-cells [26]; however, in an ascending aortic aneurism model, TSP-1 binding to CD47 was important, but alone was not sufficient for regulation of the immune response and monocyte migration [27]. This supports that TSPs work through different pathways to regulate multiple aspects of the immune response. It is likely that TSP-1 and TSP-2 modulate different aspects of the inflammatory response, as during excisional wound repair TSP-1 expression peaked very quickly, while TSP-2 expression peaked later in the repair process [28]. Although how these roles are distinct is currently unclear.

While TSP-1 and TSP-2 inhibit inflammation, TSP-4 may be pro-inflammatory. Transaortic constriction (TAC) to induce pressure overload heart dysfunction was performed on TSP-4 KO and WT mice. The TSP-4 KO mice displayed lower levels of mRNA for several inflammatory-associated proteins: intracellular adhesion molecule 1 (ICAM1), chemokines, nuclear factor κ -light-chain-enhancer (NF- κ B), and CD50 [29]. At baseline, there was no difference between the KO and WT mice. This suggests that TSP-4 is necessary for an appropriate inflammatory response with stress, but not maintaining it at baseline. This was similar to what the same group observed previously in atherosclerotic lesions [30], where they implicated TSP-4 via its ability to bind β_2 and β_3 integrins on macrophages (Figure 2).

Matrix Remodeling and Fibrosis

Among the cellular damage in the acute post-infarct period, there is significant degradation of interstitial collagen, which contributes to early adverse remodeling, including wall thinning [31]. Furthermore, the early inflammatory response can further degrade the

extracellular matrix (ECM) [32]. Timely development of a stable scar that can structurally replace the damaged tissue is necessary to avoid continued remodeling that progresses to heart failure [33]. On the other hand, an unchecked fibrotic response could lead to wall thickening and progress to maladaptive remodeling [34]. This ying-yang balance is maintained in part by Matrix Metalloproteinases (MMPs) that break down the matrix and Tissue Inhibitors of Metalloproteinases (TIMPs) [35, 36]. From a cellular standpoint, much of these mechanisms are driven by fibroblasts that differentiate into myofibroblasts in the post-ischemic environment [37].

TPS-2 knock-out mice experienced 90% mortality within 48 hours post-MI in TSP-2 [38]. This high mortality arose from cardiac rupture due to altered collagen content, suggesting that TSP-2 is essential in maintaining extracellular matrix integrity immediately following the infarct [39]. Unfortunately, this data was only ever presented as an abstract, and no follow up study ever clarified whether this was due to changes collagen formation or degradation. In other settings, there is supporting evidence that TSP-2 is necessary for a proper fibrotic response to create stable matrix. Angiotensin II (AngII) infusion induces cardiomyopathy, and in a rat model, TSP-2 expression was significantly higher in those rats that went on to failure. In TSP-2 knock-out mice, AngII resulted in 70% mortality from cardiac rupture within four days (all of the WT mice survived the four-week protocol) [40]. Those that survived exhibited an 80% decrease in fibrosis. This was mostly likely due to TSP-2's regulation of MMPs, as the knock-out mice exhibited a 120% and 390% increase in MMP2 and MMP9 activity, respectively. In cardiac cell allografts, TSP-2 knock-out hearts also experienced much less fibrosis compared to WT mice [41], and in human transplanted hearts, TSP-2 (and TSP-1) strongly correlated to the amount of collagen expression [42].

TSP-1 exhibited a faster time course of expression compared to TSP-2 in wound healing [28]. Nonetheless, its ability to activate TGF β , even in the inflammatory stages, has important ramifications in the later matrix remodeling stage. Myofibroblasts, which transdifferentiate from fibroblasts, are necessary to the fibrotic response due to their ability to generate collagen [43]. TGF β is necessary for this transdifferentiation [44], and a peptide antagonist of TSP-1's ability to activate TGF β prevented cardiac fibrosis [45]. In a TAC pressure-overload induced HF model, TSP-1 knock-out mice showed impaired TGF β -SMAD3 signaling and failed to transdifferentiate fibroblasts to myofibroblasts [46]. Without inducement to myofibroblasts, these cells exhibited significantly reduced collagen synthesis.

There is in vitro evidence that TSP-1 can bind to collagen V and fibrinogen [47, 48] and accelerate fiber growth and trifunctional branching units for fibrin intermediates [49]. The role of this direct interaction between TSP-1 and the collagen network is currently unclear, however. In addition, TSP-1 knock-out mice had significantly increased MMP9 activity after TAC [46], as was observed with TSP-2 knock-out mice with AngII infusion [40]. Thus, TSP-1 can maintain matrix integrity via regulation of MMPs [50, 51], similar to TSP-2 (Figure 2).

TSPs can affect MMP activity both directly and indirectly. Both TSP-1 and TSP-2 can directly bind MMP2 and MMP9 through their Type 1 Repeats (Figure 1) [52], although this binding doesn't directly inhibit it [53]. Instead, the complex interacts with low density

lipoprotein receptor related protein (LRP) which mediates its endocytic clearance [54]. Transcriptionally, TSPs regulate MMP9 by activating Activator protein-1 (AP-1), a positive transcriptional regulator of MMP9 [55]. Indirectly, in the TSP-1 knock-out mice, TAC increased MMP3 in addition to MMP9 [46], and MMP3 is a very efficient activator of MMP9 [56]. Lastly, TSPs can act through their activation of TGF β , which can reduce MMP transcription [57].

In the same study that observed lower inflammation in TSP-4 knockout hearts with TAC, there was an increase in fibrosis [29], suggesting that TSP-4 affects these two post-MI stages independently. The increased fibrosis was from an increase in mRNA and protein levels of the fibril-forming collagens I, II, III, and V, which TSP-4 can directly bind to [58]. This suggests that TSP-4 has a more direct role on the ECM remodeling process [59], but what this role is and the mechanism behind it are currently unclear. Upstream, TSP4 expression is regulated by the transcription factor krüppel-like factor 6 (KLF6) in order to regulate cardiac fibrosis [60] (Figure 2).

Revascularization/Angiogenesis

TSP-1 and TSP-2 activate apoptosis in endothelial cells at the microvascular level [61, 62]. The evidence supporting the latter comes predominantly from *in vitro* cancer literature. The apoptotic effect leads to inhibition of endothelial tubule formation with the consequent antiangiogenic effect. *In vitro*, both matricellular proteins have been shown to interact with the transmembrane glycoprotein CD36 [62–67], and it appears that it is mainly through this receptor that the antiangiogenic signals are triggered. Most of the studies that have addressed this come from basic biology and cancer groups. Dawson and others have shown that antibodies against CD36 inhibited the migration of human endothelial cells and tube formation [62]. Furthermore, in the same study, transfection of CD36-deficient human umbilical vein endothelial cells with a CD36 expression plasmid caused them to become sensitive to TSP-1 of their migration and tube formation. Additional motifs within the TSRs (Type I repeats, see Figure 1), implicated in heparin-binding activity, also contribute to antiangiogenic activity [68].

CD36 is a multifunctional 88kDa glycoprotein with two small cytoplasmic domains at its amino and carboxyl termini. TSP binding involves a short region of the extracellular domain [63, 69]. Inhibition of angiogenesis by TSP-1 depends on residues in the carboxy-terminal cytoplasmic domain and modulates the VEGF receptor, as was shown by Primo et al. in cultured human endothelial cells [70]. Ligation of CD36 by TSP-1 or TSP-2 results in intracellular activation of Src family kinases, fyn or yes and phosphorylation of caspases and JNK leading to apoptosis [64]. Binding of TSP-1 to CD36 also increases expression of death receptors and Fas ligand, driving endothelial cells to apoptosis [71] (Figure 3). Cell cycle progression and MAP kinase signaling in human microvascular endothelial cells are also limited by a nonapoptotic mechanism involving association of the carboxy-terminal region of TSP-2 and the VLDL receptor [72].

Both TSP-1 and TSP-2 anti-angiogenic effect has aroused interest in these proteins as a possible therapeutic strategy to block tumor angiogenesis [73]. TSP-1^{-/-} mice have

increased blood vessel density in cardiac and skeletal muscle when compared to littermate WT controls [74]. One antiangiogenic mechanism of TSP-1 is by promoting clearance of VEGF. Also, increased MMP2 and MMP9 activity in the extracellular space of TSP-1^{-/-} and TSP-2^{-/-} mice affects both cell adhesion and angiogenesis [75, 76]. In TSP-2^{-/-} mice, increased MMP-2 activity leads to degradation of tissue transglutaminase, decreased integrin activity and weaker collagen fibrils [77].

TSP-4, on contrary, has been shown to be proangiogenic. Angiogenesis is decreased in TSP-4^{-/-} mice compared with wild-type mice [78]. In this study TSP-4 was detected in the lumen of the growing blood vessels. Mice expressing the P387 TSP-4 variant, a SNP form which was previously associated with coronary artery disease and found to be more active in its cellular interactions, displayed greater angiogenesis compared with the more common A387 form. Lung endothelial cells (EC) from TSP-4^{-/-} mice exhibited more adhesion, migration, and proliferation when compared with EC from wild-type mice. In addition, recombinant TSP-4 promoted proliferation and migration of EC. Integrin α_2 and gabapentin receptor $\alpha_2\delta$ -1 were identified as the plausible receptors involved in these effects. Whether all the findings from the studies mentioned above can be extrapolated to post MI remodeling and other cardiac conditions still remains unknown. In our lab, we have not seen any difference in capillary formation after 3 weeks of TAC in hearts from TSP4^{-/-} and WT mice (Cingolani OH; unpublished data).

ADAMTS-7 is a member of the disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family [79] and was recently identified to be significantly associated genome-wide with angiographic coronary artery disease [80]. It has been previously demonstrated that ADAMTS-7 promotes vascular smooth muscle cell migration and post injury neointima formation via degradation of the matrix protein Cartilage Oligomeric Matrix Protein (COMP or thrombospondin-5) [81]. Recent work from these authors showed that ADAMTS-7 inhibits re-endothelialization by degrading TSP-1 [82]. The authors hypothesized that the degradation creates a bio-active TSP-1 peptide with potent EC recovery inhibition, similar to previous work showing that ADAMTS-1 produces TSP-1 and TSP-2 polypeptides with anti-angiogenic effects [83]. Other extracellular proteases can break down TSPs, such as thrombin, cathepsins, leukocyte elastases and plasmin [84]. These proteases can produce unique fragments, and TSP-1 and TSP-2 fragments can induce anti-angiogenic properties more effectively than the whole protein [68]. It is not currently known what role these fragments might play in the myocardium post-MI, however.

Heart Failure

HF often occurs secondary to pressure or volume overload, or a combination of both, as in the case of MI. The thrombospondins continue to play a role in the post-MI period and transition to failure, although their precise mechanisms remain to be elucidated. The thrombospondin proteins are upregulated across a wide variety of heart failure etiologies. Pressure overload quickly increased TSP-1 and TSP-4 expression [46, 85], diabetic cardiomyopathy resulted in an increase in TSP-1 [86], AngII-induced HF increased TSP-2 expression [40], hypertensive HF caused a 2-fold increase in TSP-1 [85] and a 3-fold

increase in TSP-4 [87], and volume overload saw an enormous increase in TSP-4 mRNA [88].

When the individual thrombospondin proteins are genetically removed, animals are more susceptible to HF. Removing TSP-1 increased mortality within 3-days of TAC [46], TSP-2 KO mice experienced a >5-fold increase in mortality from age-induced cardiomyopathy [89], and genetic deletion of TSP-4 caused a drastic increase in post-TAC mortality by 1 week [90]. In many instances, the mechanisms involved are similar to the post-MI remodeling heart, including inflammation [89] and fibrosis [40]. There are a number of instances, however, where the TSP proteins act in distinct ways (Figure 4).

The anti-cancer drug doxorubicin (DOX) causes dose-dependent cardiotoxicity, which can result in HF. TSP-2 KO mice were significantly more susceptible to HF with DOX treatment [91]. In addition to the known effects on the matrix (here involving MMP2), the KO mice experienced increased cardiomyocyte apoptosis linked to Akt signaling. Akt is downstream of the TSP receptor CD47/IAP and is protective, and interestingly, Akt activity is reduced even in sham operated TSP-2 KO mice [91]. Altered Akt survival signaling was also observed in age-induced cardiomyopathy with TSP-2 KO [89]. We recently described that TSP-4 also seems to play a protective role with respect to cardiac aging and exercise performance [92].

The beta-blocker metoprolol can slow or reverse cardiac remodeling [93, 94]. Both TSP-1 and TSP-4 mRNA increased during post-MI remodeling and decreased with metoprolol treatment, but only TSP-4 correlated with hypertensive heart failure pre-and post-metoprolol treatment [95]. This suggests that while both TSP-1 and TSP-4 are important during post-infarct remodeling, only TSP-4 is involved in remodeling associated with hypertensive heart failure. This disagrees with other forms of heart failure, in which TSP-1 increases (and is regulated by miR-18 and miR-19 [96]). TSP-1's precise role in heart failure, and how much it depends on the etiology of heart failure, remains to be determined.

Our group previously found a very acute role for TSP-4 with pressure overload. After 30 minutes of TAC, in which WT animals responded to the increase in afterload with increased contractility, there was a decline in function in TSP-4 null mice [97]. Given the rapid deterioration of function with increased preload, we studied the slow-force response, also called the Anrep Effect [98], in WT and TSP-4 null mice. The slow-force response (SFR) to stretch immediately follows the near-instantaneous Frank-Starling mechanism, and further increases force by roughly 15–20% over 15 minutes. After a normal Frank-Starling response, the SFR was absent in TSP-4 null trabeculae, which exhibited a slight *decrease* in force (Figure 5). Moreover, several elements of the SFR signaling cascade, including ERK1/2 phosphorylation [99], Akt phosphorylation [100], and an increase in the intracellular calcium transient [101], were absent in the TSP-4 null after 15 minutes of TAC or stretch. Isolated myocytes exposed to stretch, however, exhibited the SFR in both WT and TSP-4 null mice, suggesting either the ECM or non-myocyte cells mediate the effect.

TSP-4 also interacts with the endoplasmic reticulum (ER) stress response via reduced protein synthesis and increased degradation of damaged/misfolded proteins. The ER stress

response (or unfolded protein response) is necessary for normal cellular maintenance, but in heart failure can trigger more severe and damaging pathways like apoptosis [102, 103]. In the study by Lynch *et al.*, the absence of TSP-4 was detrimental and its overexpression was protective, and the mechanism involved modulating of the ER stress response [90]. Like a swiss-army knife, the TSP proteins seem to have a binding tool for any need, and here TSP-4 had a binding domain for an ER transcription factor. By binding to Activating transcription factor 6 α (Atf6 α), TSP-4 was able to expand the ER and increase its ability to adapt to injury. This myocyte TSP-4 response appears to be at least partially regulated by myocyte TGF β [104].

Other mechanistic roles for the TSP proteins in heart failure likely exist. These roles are likely important during the progression to heart failure as well, since the post-MI progression to HF is not a series of discrete steps, but a continuously altering environment where these signals have inertia. Thus, these roles for TSP don't suddenly appear in HF, but are also responsible for the progression to it.

Human data and Therapeutic Opportunities

Thrombospondins have also been associated with the incidence of premature heart attacks in humans, although some of the results in this area have been controversial. Single nucleotide polymorphisms (SNP) in the genes coding for TSP-1, 2 and 4 were associated with increased risk of having an acute myocardial infarction [105–109]. The latter does not seem to be directly linked with the cardiac ECM, but more with the capability these proteins have in stabilizing atherosclerotic plaques in medium to large coronary arteries. In humans, the presence of gene variations in TSP-1 and TSP-2 were not a risk factor for MI in a South Indian [105] or Chinese Han population [108], as well as in a meta-analysis [109].

In chronic heart failure patients, there was a drop in circulating TSP-1 levels [110], as well as mRNA from myocardial biopsies, which correlated positively with TGF β [111]. The change in circulating TSP-1 may be an indication of impaired angiogenesis. In human biopsies, patients with aortic stenosis were subdivided into those with diminished ejection fraction (<55%) and those with normal or supra normal ejection fraction. TSP-2 was significantly higher in those patients with diminished ejection fraction [40] and correlates with poor prognosis in heart failure with preserved ejection fraction (HFpEF) [112]. Stable HF patients who received oral anticoagulant therapy (acenocoumarol) had lower plasma TSP-1 levels than patients with similar degrees of HF who did not receive anticoagulant treatment, suggesting that acenocoumarol could facilitate angiogenesis (with better myocardial oxygen delivery) through diminishing TSP-1 [113]. TSP-4 mRNA expression increased by 3.5 times in failing humans [114], although to our knowledge, there are no studies in humans addressing whether this increase is detrimental or beneficial. In an attempt to merge the research findings in mice (where TSP-4 seemed to be cardioprotective to pressure-overload) with the fact that in humans, TSP-4 is also upregulated in athlete's skeletal muscle, we could hypothesize that this protein is required to overcome HF, and under stress conditions (i.e: pressure overload, maybe MI) reactive overexpression is essential, yet not enough at the levels achieved. Preliminary data from our lab showed that cardiac myocyte TSP4 overexpression can be accomplished in mice using adenoassociated

viral vectors (AAV9). The expression (after cardiac intracavitary injection) was diffuse, present at least 2 months after the transfection, and revealed an *in vivo* cardiac phenotype of augmented cardiac contractility (Cingolani OH; unpublished data). If this holds true, targeted overexpression could be of tremendous impact in the failing heart.

Future Directions

Many phosphorylation sites and other post-translational modifications have been identified on the TSP proteins by discovery mass spectrometry. However, none of these have been confirmed *in vivo*, and their functional relevance (if any) can only be guessed at for now. The TSPs have a wide variety of binding partners, which begs the question: how is binding regulated? The TSPs could not bind to every one of its partners simultaneously, and PTMs could be a mechanism to regulate this. Ideally, future studies would address this issue.

Very little is known about the roles of TSP-3 and TSP-5 in the heart. It was thought TSP-3 is not expressed in the myocardium [115]. However, when the post-MI heart was treated with a fragment of collagen Ia1 (called a matricryptin) generated by MMP2 and MMP9 cleavage, there was an increase in TSP-3 expression [116]. As TSP-1 and TSP-2 negatively regulate these two MMPs, they might suppress TSP-3 expression in response to stress. Whether the TSPs actually regulate each other in this manner, or if there are other examples of TSPs regulating each other need to be addressed by future studies. TSP-5 is expressed in vascular smooth muscle cells [117], and can induce chemotaxis like TSP-1 and TSP-2 can, but unlike these other TSPs, TSP-5 cannot induce proliferation [118]. Thus, there appear to be unique roles for TSP-3 and TSP-5, but what, if any, involvement they have in the transition from MI to HF remains to be discovered.

Summary

There is no doubt matricellular proteins play a key role in the transition from MI to HF, and the plot thickens with the thrombospondins, since they seem to be more than just ECM linkers. Through their many binding domains, they interact with various elements on the cell surface, ECM, and more recently appreciated, intracellular signaling cascades. The presence of all of these binding domains, however, yields an important, unanswered question: how are these binding events regulated? When CD36, CD47, and TGF β are all present, how does TSP-1 decide which to bind to? Perhaps through post-translational modifications, bio-active cleavage products, spatial control, or merely whichever is more present.

Both TSP-1 and TSP-2 are anti-inflammatory, pro-fibrotic, and anti-angiogenic, while TSP-4 behaves almost exactly opposite, exerting pro-inflammatory and pro-angiogenic effects, agreeing with the other TSPs only in its pro-fibrotic effects. These simplifications, however, gloss over the complex tuning of these effects the TSPs provide. For example, TSP-1 isn't merely anti-inflammatory; it provides a spatial barrier that restricts the inflammatory response to only the region that requires it [16]. The expression patterns of the TSPs appear to be tightly controlled, both spatially and temporally. This constantly changing environment makes therapeutic targeting challenging, however, as what may be beneficial acutely in the border zone, may be harmful later on in the infarct zone. Nonetheless, the

central roles the TSPs play make them attractive targets for slowing or stopping the progression from a MI to HF.

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Highlights

- Advances in acute MI care have created a population at high risk of heart failure
- The thrombospondin (TSP) family of secreted glycoproteins is upregulated post-MI
- TSPs are involved in post-MI inflammation, matrix remodeling, and angiogenesis
- Myocyte hypertrophy, apoptosis, and contractile function are also affected by TSPs
- The TSPs represent an attractive target for controlling the post-MI transition to HF

Figure 1. Structure of the two thrombospondin (TSP) subgroups

Subgroup A form homotrimers and consist of TSP-1 and TSP-2, while Subgroup B form homopentamers and consist of TSP-3, TSP-4, and TSP-5 (COMP). Subgroup A has domains that bind to CD36 and inhibit MMPs. The N-terminal domains tend to be family member specific, while the CTD has high homology between the family members. NTD: N-terminal domain (specific to each family member), vWF-C: von Willebrand factor C-type domain, MMP: matrix metalloproteinase, EGF: epidermal growth factor, CTD: C-terminal domain.

Figure 2. The TSPs regulate inflammation and fibrotic matrix remodeling post MI

As matricellular proteins, the TSP family interacts with multiple different cells types in the myocardium to induce their effects. TSP-1 and TSP-2 affect remodeling through their interactions with transforming growth factor β (TGF β) and its signaling in fibroblasts, matrix metalloproteinases (MMPs), and interactions with CD47 on T-cells. TSP-4 is suggested to be pro-inflammatory (opposite of TSP-1/2) through interactions with macrophages. Moreover, TSP-4 can inhibit collagen production (possibly through direct binding and interaction), and this may involve krüppel-like factor 6 (KLF6) transcriptional regulation of TSP-4.

Figure 3. TSP1 and TSP2 are anti-angiogenic, while TSP-4 is pro-angiogenic

Both TSP-1 and TSP-2 provide their anti-angiogenic properties by binding to CD36 via their Type I repeats. Subsequent signaling through Src, Fyn, JNK, Fas, and others results in apoptotic/anti-angiogenic effects. While it is known that TSP-4 has pro-survival and pro-angiogenic capabilities, opposite those of TSP-1 and TSP-2, the mechanisms are less clear. Lacking the Type I repeats of the Subgroup A TSP trimers, TSP-4 doesn't possess the CD36 binding domain. Recent evidence suggests the integrin α_2 and gabapentin receptor $\alpha_2\delta-1$, but beyond that the signaling is currently unknown.

Figure 4. Recent work has identified several roles for TSPs once the heart has transitioned to heart failure

The effects the TSPs have on matrix remodeling and inflammation are still important in heart failure. However, they also regulate a number of pathophysiologically important elements within the cardiac myocyte, which exhibits hypertrophy, apoptosis, and contractile dysfunction with HF. However, our knowledge of the mechanistic function of each of the TSPs in the HF myocyte is still incomplete.

Figure 5. Thrombospondin-4 is necessary for the heart's response to acute changes in volume/length

The slow force response occurs when a myocyte experiences stretch, often on the order of 5–6%. The acute phase of the pressure overload also stretches the myocytes. In normal mice, stretch causes a nearly instantaneous increase in force via the Frank-Starling (FS) mechanism, and then a steady increase in force over the following 15 minutes known as the slow-force response, or Anrep Effect. In the thrombospondin-4 knockout (TSP-4 KO) mice, the FS mechanism is intact, but the slow-force response is absent. In fact, force decreases slightly in response to stretch (red line). Figure adapted from [97].

Table 1

Thrombospondin binding partners

Protein	ECM	Cell Surface	Signaling Molecules
TSP-1	MMP2, MMP9, collagen V, fibrinogen, fibronectin	CD36, CD47, $\beta 1$, $\beta 3$ integrins	TGF- β VEGF, Ca^{2+}
TSP-2	MMP2, MMP9	CD36, CD47	Ca^{2+}
TSP-4	Collagens I, II, III, V	$\beta 2\beta 3$ integrins	Atf6 α