Editorial

Maintaining Matrix Composure Under Stress

April Stempien-Otero

Increased left ventricular wall stress precedes the development of heart failure in multiple myocardial pathologies. Increased wall stress can occur in many forms. It may occur globally from increased in pressure throughout the ventricle such as in hypertension or aortic stenosis or regional as in myocardial ischemia where scar or dysfunctional myocardium creates imbalances in stress between areas of normal and hypo-perfusion.[1] At a cellular level, loss of myocytes throughout the myocardium due to toxins or myocarditis leads to replacement fibrosis altering the strain properties of the tissue and increasing wall stress.[2] Similarly, genetic alterations in sarcomeric structure can predictably change stress locally resulting in compensatory hypertrophy or dilation.[3]

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As the heart is exposed to continuous and ever-changing mechanical forces, it is not surprising that it has adapted many strategies to deal with changes in wall stress. Although acute intermittent increases in stress with exercise lead to adaptive hypertrophy, chronic wall stress in the pathological conditions above additionally promotes activation of fibroblasts and increased inflammation. These changes, which in animal models can be seen before the development of systolic and or diastolic dysfunction, are associated with local activation of profibrotic factors, such as TGF-β (transforming growth factor-beta) and angiotensin 2. As fibrosis causes increases in both tissue strain and wall stress, it has been hypothesized that these responses are maladaptive and lead to a vicious cycle of pathology ending in heart failure.[4]

The data presented by Russo et al[5] counter that long-standing paradigm and shed light on the complexity of the heart's response to stress. They focused on the role of TGF-β signaling in a murine model of global pressure overload–transverse aortic constriction (TAC). Focusing on the mechanisms by which TGF-β regulates myocardial remodeling in response to stress is a well-trod path. A myriad of studies have demonstrated the profibrotic properties of TGF-β signaling pathways in the heart.[6] However, global knockouts have shown both beneficial and deleterious effects of manipulating this pathway, making therapeutic targeting elusive. To dissect the effects of TGF-β

specific to activated fibroblasts in response to stress, Russo et al[5] generated a conditional knockout of Smad3 (mothers against decapentaplegic homolog 3), a downstream effector of TGF-β signaling (referred by the authors as FS3KO mice) using a Periostin-Cre driver. A previous study of a global knockout of Smad3 subjected to TAC resulted in decreased fibrosis, but excess mortality was seen in this model.[7] One would, therefore, expect that Smad3 knockout specific to fibroblasts would provide a decrease in fibrosis without the other deleterious effects.

Surprisingly, the FS3KO mice had no substantial decrease in fibrosis at 7 or 28 days following TAC. Furthermore, there was decreased systolic function compared with control genotypes treated with TAC. The authors are to be commended for performing cell and matrix specific analysis to understand this unexpected finding. Their data demonstrate that absence of Smad3 signaling resulted in increased matrix fragmentation, increased accumulation of cardiac macrophages, and increased cardiomyocyte cytolysis early following TAC. Furthermore, there was a change in macrophage phenotype within the FS3KO mice post-TAC with a shift towards more M1 macrophages. Expression analysis of isolated cardiac fibroblasts showed no difference in expression of profibrotic factors or matrix components but rather a significant increase in MMP (matrix metalloproteinase)-8 expression, a known collagenase. The authors validated the role of MMP-8 in the phenotypic changes with the use of an MMP-8 inhibitor. MMP-8 inhibitor–treated FS3KO TAC mice had less reduction in ejection fraction and less inflammatory M1 macrophages compared with vehicle-treated FS3KO TAC mice. However, MMP-8 inhibition did not alter rates of myocytolysis or total macrophages accumulation. Importantly, control mice treated with the MMP-8 inhibitor also had reductions in ejection fraction.

These seemingly contradictory results of the MMP-8 inhibitor studies—beneficial in one condition/deleterious in another—are consistent with literature in which over or underexpression of proteinases assumed to degrade matrix and reduce fibrosis fail to achieve the predicted end point. Furthermore, these data highlight the limitations of using this strategy therapeutically. More promising from a therapeutic stand point is data from Russo et al[5] demonstrating an increase in circulating levels of a cleaved matrix peptide-Pro-Gly-Pro in FS3KO mice. Although the existence of matrikines, matrix cleavage products with biologic activity, has been described for many years, catching one in the act in an in vivo model has been challenging. The linkage between this peptide and increased inflammation is a tantalizing hypothesis. Although a broader proteomic screen of circulating proteins is necessary to determine the specificity of this finding, targeting an active peptide for a set period of time during increased wall stress is therapeutically attractive.

It is important to note that Russo et al[5] are not the first group to test the effect of pressure overload on a fibroblast-specific knockout of Smad3. Khalil et al[8] used an inducible MerCreMer system to driving Periostin-Cre to delete multiple components of this pathway including Smad3, Smad2, TGF-β1, and TGF-β2 in mice before TAC. In this study, deletion of Smad3 in periostin expressing fibroblasts resulted in the reduced fibrosis at 12 weeks. A comparison of the 2 studies is revealing in what can (and cannot) be learned from pre-clinical animal models. Both studies used periostin to target activated fibroblasts. Although there are strong data indicating an association of increased periostin expression with activated fibroblasts in injured myocardium, periostin is also expressed at lower levels in cardiac fibroblasts in normal hearts.[9] Therefore, in both models, it is unknown what specific fibroblast populations underwent Cre recombination of Smad3, and at what time point. In Russo et al,[5] it is likely that Smad3 was completely deleted in resting fibroblasts before TAC due to the constitutive nature of the construct as opposed to Khalil et al[8] who initiated the deletion with tamoxifen treatment just 48 hours to TAC. Furthermore, the analysis of fibroblast gene expression was different between the studies. Khalil et al[8] analyzed only Smad3 null fibroblasts-labeled by a fluorescent reporter; these data showed dramatic reductions in matrix gene expression. In contradistinction, Russo et al[5] analyzed gene expression from all cardiac fibroblasts and found no differences, implying that other resident fibroblasts may compensate for loss of matrix formation.

In summary, Russo et al[5] have demonstrated that TGF-β pathways can provide adaptive responses to increased wall stress by restraining proteinase production in cardiac fibroblasts and thus preserving matrix integrity. In combination with the study of Khalil et al,[8] it is intriguing to hypothesize that this pathway is adaptive in situations of increased wall stress (ie, during prolonged exercise) but becomes maladaptive under chronic stress. These data support other studies that the role of TGF-β in the heart is dependent not just on cell phenotype but potentially on cell subtypes similar to its role in the immune system. It is truly pleiotropic molecule whose beneficial and detrimental effects are highly situational. Understanding its function has provided substantial mechanistic information on the response of the heart to stress, and with time will hopefully yield a downstream target for safe and effective antifibrotic therapies.

Disclosures
None.

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KEY WORDS: Editorials ■ heart failure ■ hypertrophy ■ inflammation ■ macrophages