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EDITORIAL COMMENT

# Putting the Heat on Cardiac Fibrosis

## Hsp20 Regulates Myocyte-To-Fibroblast Crosstalk*

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Fibrosis is a wound-healing process that is triggered by tissue injury or stress. Cardiac fibrosis is associated with adverse outcomes in several forms of heart failure (HF), including HF with reduced ejection fraction, HF with preserved ejection fraction, and genetically driven cardiomyopathies (1,2). Although the increased extracellular matrix (ECM) deposition that accompanies fibrotic responses may acutely serve to stabilize a focal area of myocardial damage, excessive, diffuse, or chronic activation of fibrosis can be deleterious to long-term cardiac function and patient survival. For example, fibrosis can increase the passive stiffness of the myocardium, which contributes to diastolic dysfunction (3,4), and can disrupt electrical conduction in the heart, which causes arrhythmias and sudden cardiac death (5). Unfortunately, despite the well-accepted roles of fibrosis in cardiac dysfunction, no targeted antifibrotic drugs for the heart exist. Thus, it is crucial to understand the fundamental mechanisms that drive cardiac fibrosis so that novel approaches to thwart this pathogenic process can be discovered.

Resident fibroblasts in the heart are major contributors to cardiac fibrosis (6,7). In response to stress, these cells undergo a cell state transition to become activated fibroblasts, sometimes referred to as myofibroblasts, which produce high levels of ECM. Inflammatory cues from dead myocytes, leukocytes, vascular cells, and resident fibroblasts themselves have historically been viewed as the major drivers of fibroblast activation in the heart. However, there is a growing body of evidence to support a role for myocyte-derived secreted factors in the control of the cardiac fibroblast activation (8,9). In this issue of *JACC: Basic to Translational Science,* Gardner et al. (10) reveal a function for heat shock protein 20 (Hsp20) in the regulation of pro-fibrotic cardiomyocyte-to-fibroblast crosstalk.

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Hsp20 is a member of the small heat shock superfamily of proteins that function as chaperones to prevent protein misfolding through adenosine triphosphate–independent processes (11,12). Over the last decade, several studies have demonstrated cardioprotective functions of Hsp20. Work by Chu et al. (13) and Fan et al. (14) established that cardiomyocyte Hsp20 levels and phosphorylation at serine-16 were increased by β-adrenergic stimulation, which resulted in protection against apoptosis. Subsequently, they discovered that transgenic mice with cardiomyocyte-specific expression of Hsp20 were protected from ischemia-reperfusion injury (15). The protective effects of Hsp20 in the heart were corroborated by other groups using distinct cell-based and in vivo models of cardiac stress (16). Furthermore, cell culture studies that used Hsp20 derivatives harboring a phosphomimetic or a non-phosphorylatable amino acid in place of serine-16, and S16D and S16A, respectively, implicated protein kinase A (PKA)– or protein kinase D–mediated phosphorylation of this site as a beneficial signaling event in cardiomyocytes (16).

Paradoxically, in the current study, Gardner et al. (10) showed that cardiomyocyte-specific expression of Hsp20-S16D in mice led to systolic dysfunction and 100% mortality in <1 year. In contrast, there were no

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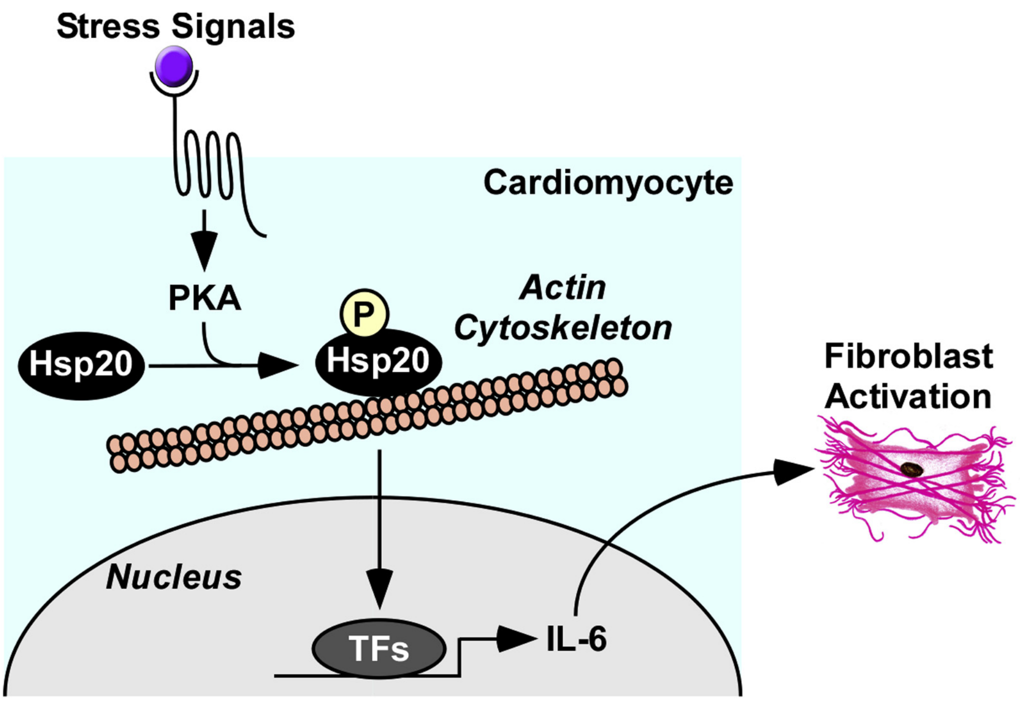
**FIGURE 1** **Model for Hsp20-Mediated Cardiomyocyte-to-Fibroblast Crosstalk**



Stress signals lead to protein kinase A (PKA)–mediated phosphorylation of heat shock protein 20 (Hsp20) in cardiomyocytes. Phospho-Hsp20 associates with the actin cytoskeleton and transmits activating signals to nuclear transcription factors (TFs) that stimulate production of interleukin (IL)-6. IL-6, secreted from cardiomyocytes, functions in a paracrine manner to stimulate resident cardiac fibroblasts.

deleterious effects of transgene-mediated expression of non-phosphorylatable Hsp20-H16A in the heart. Hsp20-S16D transgenic mice exhibited significant interstitial fibrosis before evidence of myocyte apoptosis, which led the investigators to postulate that phospho-Hsp20 could be triggering reactive interstitial fibrosis through paracrine activation of resident fibroblasts. Consistent with this notion, exposure of cultured cardiac fibroblasts to medium from cultured cardiomyocytes ectopically expressing Hsp20-S16D, but not Hsp20-S16A, led to a modest increase in fibroblast activation markers. The investigators went on to show that Hsp20-S16D promotes production and secretion of interleukin-6 (IL-6), which has the capacity to stimulate cardiac fibroblasts in vitro and in vivo. A 1-month treatment with a neutralizing antibody against IL-6 was found to block pathological cardiac fibrosis in Hsp20-S16D transgenic mice.

The current findings further establish the importance of myocyte-derived paracrine signaling in the control of fibroblast activation in the heart, and suggest novel approaches for therapeutically targeting cardiac fibrosis based on IL-6 inhibition or altering Hsp20 phosphorylation and/or function. Although inhibiting IL-6 signaling has yielded contradictory results in murine models by either blunting or exacerbating cardiac disease, a recent phase 2 clinical trial demonstrated that tocilizumab, a humanized monoclonal antibody against the IL-6 receptor, reduced inflammation and cardiac damage in patients post-myocardial infarction (17,18). In the future, cardiac cardiac magnetic resonance, which is the current gold standard modality for noninvasive evaluation of cardiac fibrosis, could be used to evaluate the ability of tocilizumab and other IL-6 targeted therapies to reduce ECM deposition in the heart.

Regarding Hsp20, most efforts to date have focused on enhancing phosphorylation of this chaperone as a therapeutic strategy for HF. Hsp20 is found in multiprotein complexes that include phosphodiesterase-4 (PDE4), which degrades cyclic adenosine monophosphate and thereby dampens PKA-mediated phosphorylation of substrates, including Hsp20. Peptide disrupters of the Hsp20–PDE4 interaction have been shown to increase Hsp20 phosphorylation and block cardiomyocyte hypertrophy and fibrosis, which suggests that, counter to the conclusions of the current study, Hsp20 phosphorylation is cardioprotective (19,20).

Because of potential translational significance of Hsp20 phosphorylation, it will be critical to extend the findings of Gardner et al. (10) to further address the question of whether this post-translational modification is beneficial or detrimental to the heart. The answer likely lies somewhere in the middle, with the cost-to-benefit ratio of Hsp20 phosphorylation being determined by factors such as the stoichiometry and duration of the phospho-modification. Because Hsp20-S16D was expressed in >10-fold excess of the endogenous protein, most of the pool of this chaperone in cardiomyocytes represents the phospho form. It is possible that balancing the amount of transgene-produced S16D versus endogenous Hsp20 to more closely match physiological levels of phospho-Hsp20 will yield distinct effects, which may be salutary. Additionally, implementation of an inducible transgene system that enables temporal modulation of S16D expression acutely following myocardial infarction could uncover the protective effects of Hsp20 phosphorylation. This latter system would enable investigators to address the possibility that acute increases in phospho-Hsp20 exert beneficial effects in the context of a pathogenic insult, but disrupt cardiac homeostasis in the absence of stress.

It will also be important to determine if the discrepancy between the current findings and previous work, which suggested favorable consequences of Hsp20 phosphorylation, is due to the use of the S16D construct. Aspartic and glutamic acid are frequently used to mimic the negative charge of a phospho group, but these substitutions do not always recapitulate the consequences of site-specific phosphorylation (21). Knock-in mice harboring an alanine codon for amino acid 16 in the endogenous Hsp20 locus should be particularly informative.

Additional investigation of the mechanisms by which Hsp20 controls IL-6 expression in cardiac muscle also has the potential to guide translational efforts. Previous studies have demonstrated that β-adrenergic receptor signaling in cardiomyocytes leads to PKA-dependent recruitment of Hsp20 to the actin cytoskeleton, which is coupled to enhanced cellular contraction (14). Presumably, the actin-associated pool of phospho-Hsp20 conveys signals to nuclear transcription factors that control IL-6 gene expression (**Figure 1**). Details about the molecular basis for this cytoskeleton-to-nucleus communication in cardiomyocytes could reveal regulatory nodes that could be manipulated to blunt the transcriptional network that governs pathogenic cardiomyocyte-to-fibroblast crosstalk. As alluded to by the investigators, phospho-Hsp20 might also function within the cardiomyocyte nucleus to stimulate IL-6 gene expression.

In summary, the compelling study described by Gardner et al. (10) has advanced our understanding of the mechanisms that control fibrosis of the heart and has shed light on possible avenues for therapeutic intervention, while concurrently uncovering new and exciting questions. Answers to these questions will undoubtedly be forthcoming as investigators continue to put the heat on the problem of cardiac fibrosis.

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