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EDITORIAL COMMENT

Cut the YAP

Limiting Fibrosis in Pathologic Cardiac Remodeling*

Eric M. Small, PhD, Alan C. Brooks, MD, PhD

Cardiovascular disease accounts for a growing portion of the global health care burden and is a leading cause of hospitalization (1). Despite guideline-directed medical therapy and implantable devices that have addressed some of the therapeutic and monitoring needs in heart disease, progression to heart failure remains a major cause of morbidity and mortality across the world. Incomplete understanding of cardiovascular disease mechanisms remains a critical barrier to the advancement of more effective treatment options for patients with heart failure. One emerging area of translational research involves defining the contribution of the extracellular matrix (ECM) to normal cardiac physiology and pathological remodeling. At a cellular level, resident fibroblasts are believed to be the primary source of ECM molecules, such as collagens and fibronectin in health and disease (2). Heart failure is characterized by the accumulation of activated cardiac fibroblasts, also called myofibroblasts, that acquire a contractile phenotype and deposit excessive amounts of ECM within the cardiac interstitium. In heart disease, ECM deposition by myofibroblasts is initially an adaptive response to cardiac insult that may have evolved to increase structural integrity; however, persistent ECM deposition precipitates the development of a fibrotic scar that impedes ventricular compliance and serves as a substrate for arrhythmias. Antifibrotic strategies that effectively and specifically target fibroblast activation and cardiac scarring will fill a critical unmet need for the expanding heart failure population.

Recent advances in gene editing technologies, in conjunction with genomic, transcriptomic, and proteomic platforms, have accelerated the discovery of mechanisms that underlie pathological cardiac fibrosis. During embryonic development, cardiac fibroblasts are generally believed to originate from the epicardium, a layer of mesothelial cells on the surface of the heart that are defined by the expression of *Wt1* and *Tcf21*. Genetically modified mice that expressed tamoxifen-inducible Cre-recombinase under the control of the *Tcf21* gene, in particular, allowed for fibroblast-specific gene deletion and lineage-tracing studies in murine models of heart failure (3). Such studies have expanded our understanding of the factors that underlie myofibroblast activation and fibrosis, which include biomechanical tension, canonical and non-canonical transforming growth factor−β signaling, and neurohumoral signaling. These biomechanical and signal transduction pathways preferentially converge on transcriptional programs driven by SMADs (an acronym referring to similarities between *C. elegans* "small worm phenotype" [*SMA*] and Drosophila "Mothers Against Decapentaplegic" [*MAD*] families of genes) and myocardin-related transcription factors (MRTFs), among others, that mediate myofibroblast activation (4). Many genes that encode ECM and contractile proteins harbor SMAD and MRTF responsive elements in their promoters. Although effective SMAD and MRTF inhibitors have been developed (5), the ubiquitous contributions of these transcriptional regulators to normal tissue homeostasis complicates

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the translation of tightly controlled pre-clinical findings to the clinic.

In this issue of *JACC: Basic to Translational Science*, Francisco et al. (6) established the role of the Hippo pathway transcriptional regulator, Yes1-associated transcriptional regulator (YAP), in myofibroblast activation subsequent to myocardial infarction (MI) and in response to neurohormonal activation via angiotensin II (Ang II) infusion in mice. Hippo signaling was initially discovered in a Drosophila mutagenesis screen and has since been linked to myriad pathophysiological processes as a cell contact sensor that regulates cell behavior and organ size via restraint of the cell cycle (7). Hippo signaling is active in normal tissue homeostasis, in which a series of phosphorylation events are maintained by a cytoplasmic kinase cascade that includes macrophage stimulating (Mst)1/2 and large tumor suppressor kinase (Lats)1/2. Active Hippo signaling leads to Lats1/2-dependent phosphorylation of the transcription factors YAP1 and WW domain-containing transcription regulator 1 (Wwtr1, also known as TAZ). Phosphorylated YAP and TAZ are subsequently retained in the cytoplasm where they cannot contribute to transcriptional activation. Upon disruption of cell contacts or excessive biomechanical force generation, Hippo kinase signaling is inactivated, leading to accumulation of unphosphorylated YAP and TAZ in the nucleus, where they bind to the TEA domain transcription factor 1 (TEAD, previously known as TCF13 or TEF-1) and activate TEAD target genes. The targets that are induced by TEAD and YAP/TAZ tend to be pro-proliferation and pro-inflammatory genes; therefore, regulated inactivation of Hippo signaling through biomechanical forces leads to YAP-dependent cell growth. When Hippo signaling is inappropriately inactivated, it can lead to cell and tissue dysfunction, including cancerous cell overgrowth. Targeting Hippo signaling pharmacologically or genetically is gaining considerable interest, particularly for cancer treatment or regenerative medicine approaches.

In the paper by Francisco et al. (6) in this issue of *JACC: Basic to Translational Science*, the investigators found that YAP was activated (dephosphorylated) in fibroblasts that were stimulated to become myofibroblasts with Ang II treatment via a RhoA-dependent mechanism. As expected based on previous studies, active YAP stimulated proliferation genes and accelerated the cell cycle in cardiac fibroblasts. YAP also induced myofibroblast genes, such as *Col1a1* and alpha-smooth muscle actin (*Acta2*) and increased fibroblast contractility as demonstrated by in vitro collagen gel contraction assays. Importantly, Ang II-dependent myofibroblast activation could be blocked in vitro using verteporfin, a previously established YAP inhibitor. The investigators extended their in vitro observations by deleting the *Yap1* gene in fibroblasts using *Tcf21* or *Col1a1*-driven Cre-recombinase (YAP-cKO), which revealed a diminution of fibrosis in YAP-cKO mice. Reduced fibrosis was accompanied by improved cardiac physiology after MI but surprisingly not following Ang II infusion. Therefore, it will be interesting to compare and contrast the impact of YAP on scar formation and cardiac physiology in ischemic and nonischemic cardiac remodeling.

The previously described findings are consistent with a recent study that described the precocious development of cardiac fibrosis upon disruption of Hippo signaling via fibroblast-specific *Lats1/2* deletion in mice (8). However, in contrast to direct activation of myofibroblast genes by YAP reported in that study, Francisco et al. (6) found that YAP directly induced the expression of the *Mkl1* gene (also known as *Mrtf-a)* through a TEAD binding site in the *Mkl1* promoter. Chromatin immunoprecipitation demonstrated YAP occupancy at the TEAD site in the *Mkl1* promoter, and mutation of the TEAD sites abolished YAP-dependent activity of an *Mkl1* promoter-driven luciferase reporter. Furthermore, knockdown of Mrtf-a ameliorated the induction of myofibroblast markers upon YAP overexpression. Thus, the investigators proposed that YAP stimulated myofibroblast activation secondarily through the induction of *Mrtf-a* gene expression. The Mrtf-a protein was previously shown to induce myofibroblast gene programs after MI (9).

Although the exciting findings by Francisco et al. (6) suggested a new target for antifibrotic approaches, the complexity of Hippo signaling and myofibroblast activation pathways precluded the generation of a simple mechanism of action. Additional studies are necessary to align the current findings with established mechanisms of YAP and MRTF activity. First, the investigators reported that YAP directly activated *Mrtf-a* gene expression, which they suggested was the primary mechanism of YAP-dependent fibroblast activation. However, significant alterations in *Mrtf-a* gene expression or protein abundance in pathological cardiac remodeling have not been consistently reported. Instead, *Mrtf*-dependent gene activation typically occurs via biomechanical alterations in Rho-dependent actin dynamics that lead to *Mrtf-a* nuclear accumulation (10). It remains possible that, in addition to activating *Mrtf-a* gene expression, YAP also stimulates MRTF activity via biomechanical alterations that affect actin dynamics. It will be

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interesting to further pursue the mechanisms that link Hippo and MRTF-dependent gene regulation and fibroblast activation. Second, the investigators found that YAP was at least partially responsible for stimulating cardiac fibroblast proliferation, as described in other cell systems. It remains possible that alterations in the proliferation rate that occur upon manipulating YAP levels and activity may contribute to the changes in cardiac fibrosis. Recent studies indicated that senescence and proliferation pathways might affect fibroblast activation. Although it may be difficult to separate the impact of Hippo signaling on the cell cycle from its impact on myofibroblast activation, future studies should address the relative contribution of YAP-dependent fibroblast proliferation and Mrtf-a–dependent fibroblast activation in pathological cardiac remodeling.

Collectively, the study by Francisco et al. (6) provides a potential new therapeutic strategy aimed at limiting long-term cardiac fibrosis and its adverse sequelae. Future studies should evaluate the risks and benefits of YAP inhibition, including an expanded evaluation of verteporfin in pre-clinical heart failure models. It is possible that inappropriately timed YAP inhibition may sabotage an adaptive injury response. For instance, increased incidence of cardiac rupture could be an unintended consequence of YAP inhibition during the post-MI period, although Francisco et al. (6) did not observe a rupture in the mild MI model presented in their study. Another important aspect for future investigation is the long-term implications of YAP inhibition on cardiac function. In the 4-week post-MI time point evaluated in the current study, YAP appeared to induce pathological fibrosis and to impede normal cardiac function. However, it remains to be seen whether YAP inhibition would remain protective or have unintended off-target consequences. Overall, the results presented by Francisco et al. (6) expanded the scope of pathophysiological processes affected by Hippo signaling and could accelerate the development of novel strategies aimed at preventing cardiac fibrosis in patients with heart failure.

ADDRESS FOR CORRESPONDENCE: Dr. Eric M. Small, Aab Cardiovascular Research Institute, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Box CVRI, Rochester, New York 14642. E-mail: eric_small@urmc.rochester.edu.

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