Hippo signaling does it again: arbitrating cardiac fibroblast identity and activation

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The Hippo pathway is an evolutionarily conserved kinase cascade that is fundamental for tissue development, homeostasis, and regeneration. In the developing mammalian heart, Hippo signaling regulates cardiomyocyte numbers and organ size. While cardiomyocytes in the adult heart are largely postmitotic, Hippo deficiency can increase proliferation of these cells and affect cardiac regenerative capacity. Recent studies have also shown that resident cardiac fibroblasts play a critical role in disease responsiveness and healing, and in this issue of *Genes and Development*, Xiao and colleagues (pp. 1491–1505) demonstrate that Hippo signaling also integrates the activity of fibroblasts in the heart. They show that Hippo signaling normally maintains the cardiac fibroblast in a resting state and, conversely, its inactivation during disease-related stress results in a spontaneous transition toward a myofibroblast state that underlies fibrosis and ventricular remodeling. This phenotypic switch is associated with increased cytokine signaling that promotes nonautonomous resident fibroblast and myeloid cell activation.

Hippo signaling was first uncovered in *Drosophila melanogaster* using genetic mosaic screens to search for tumor suppressors, where it was shown to mediate cell proliferation, tissue, and organ size (Xu et al. 1995; Harvey et al. 2003). The signaling pathway includes the upstream kinase Hippo, which is orthologous to mammalian Ste20-like kinases 1/2 (Mst1/2) (Fig. 1). Mst1/2 form a complex with Salvador (also known as WW45) that permits phosphorylation and activation of the large tumor suppressor homolog kinases1/2 (Lats1/2) in mammals. Lats1/2 then phosphorylate the transcriptional cofactors Yes-associated protein (Yap) and Taz, preventing their nuclear localization and association with the TEA domain family (TEAD1-4) of transcription factors (Liu and Martin 2019). Inactivation of Mst1/2 or Lats1/2 allows Yap and Taz to persist in an unphosphorylated state, permitting translocation to the nucleus, where they interact with TEAD factors to promote expression of proliferative and growth-related genes (Liu and Martin 2019). Changes in cell shape and/or tissue rigidity can also activate Yap/Taz via mechanotransduction, a critical phenomenon in organogenesis, tissue homeostasis, and regeneration where physical cues from the extracellular matrix (ECM) activate gene expression in a cell-specific manner (Fig. 1; Panciera et al. 2017).

Cardiac fibroblasts (CFs) are the main cell type that synthesize and maintain the ECM in the heart (Khalil et al. 2019). Upon activation by acute injury and/or inflammatory signals, these cells convert to a more synthetic and contractile state known as the myofibroblast that promotes tissue fibrosis and remodeling (Tallquist and Molkentin 2017). During development, CFs are derived from a layer of cells that encapsulates the heart, known as the epicardium (Acharya et al. 2012). Recent studies have shown that inactivation of the Hippo pathway by deletion of *Lats1/2* in proepicardial cells prevents formation of CFs, resulting in a grossly abnormal heart (Xiao et al. 2018). Such results could also be extrapolated to suggest that Hippo signaling should also underlie adult tissue fibroblast conversion to myofibroblasts with acute and chronic disease stimuli. Indeed, fibroblasts are activated in injured tissues by inflammatory signals and stretch, stimuli that also broadly lead to Yap and Taz nuclear accumulation (Liu et al. 2015; Piersma et al. 2015; Ramjee et al. 2017). However, direct mechanistic data linking Hippo signaling in CFs to adult disease states was lacking.

In this issue of *Genes & Development*, Xiao and colleagues use elegant mouse genetic strategies to mechanistically dissect the role that Hippo signaling plays within adult CFs from both the healthy and ischemic heart (Xiao et al. 2019). To deactivate Hippo signaling, the authors conditionally deleted *Lats1/2* genes in CFs (*Lats1/2* CKO) using a fibroblast-specific, adult inducible Cre strategy, which spontaneously activated cardiac fibrosis

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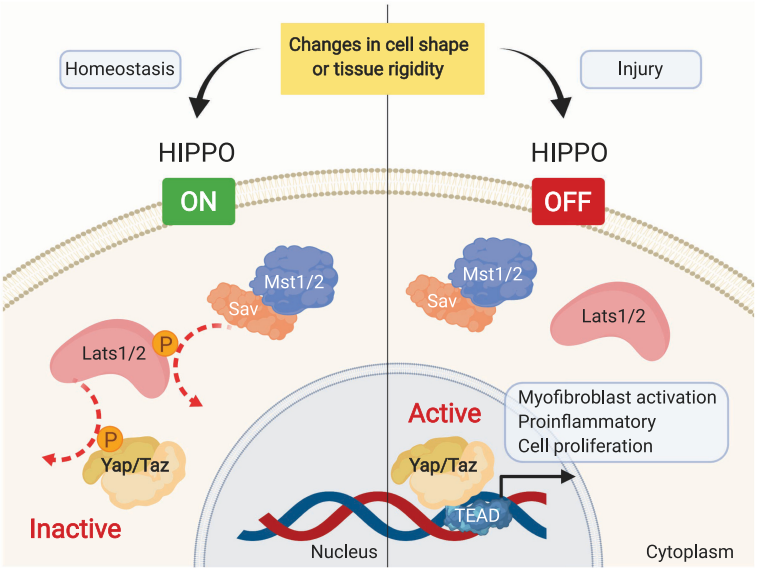

Figure 1. Hippo signaling in cardiac fibroblasts. In the adult heart, Hippo signaling is required to maintain fibroblasts in their quiescent state (*left*). Upon fibroblast activation, Hippo signaling is switched off, leading to Yap/Taz nuclear localization (*right*). Downstream from membrane signal transduction and/or stretch sensing, Mst1/2 form a complex with Salvador (Sav) to mediate phosphorylation of the kinases Lats1/2. In turn, Lats1/2 mediates phosphorylation of Yes-associated protein (Yap) and Taz, which sequesters them in the cytoplasm or leads to their degradation. Unphosphorylated Yap/Taz reside within the nucleus where they interact with TEA domain (TEAD) transcription factors to regulate genes involved in myofibroblast formation and function, as well as induction of inflammatory signals.

in the endo- and epicardial regions of the otherwise uninjured heart. Single-cell sequencing analysis revealed unique epicardial, fibroblast, and myeloid clusters in these *Lats1/2* CKO hearts. Two of the fibroblast clusters had inflammatory gene signatures, one expressed markers of activated fibroblasts, and four clusters were identified as myofibroblasts that expressed Yap/TEAD target genes. Interestingly, not all the clusters showed a loss of Lats1/2 expression due to known inefficiency in Cre-mediated recombination, which serendipitously generated an internal control for the unique gene signatures associated with *Lats1/2* deletion and constitutive Yap/Taz activity. For example, myofibroblasts had an approximately fivefold increase in gene expression for ligand-receptor pairings, with the expression of ligands known to participate in the wound-healing response. This was further supported by the increased expression of cytokines in the *Lats1/2* CKO hearts, including select chemokines (CCL12, CCL2), colony-stimulating factor 1, and tissue inhibitor of metalloproteinases-1. In addition to these fibroblast clusters, there was an emergence of seven myeloid lineage clusters unique to the *Lats1/2* CKO hearts, suggesting that these altered fibroblasts promote inflammatory signaling.

To identify other gene regulatory networks and cell states, the authors applied a bioinformatic methodology known as SCENIC (Aibar et al. 2017), which first determines genes that are co-expressed with a transcription factor and then performs motif analysis to remove indirect target genes. As expected, TEAD regulons were enriched within the myofibroblast clusters. In addition, regulons associated with endoplasmic reticulum stress and the unfolded protein response were enriched within this cluster. To further analyze Yap target genes, the authors performed HiCHIP, which demonstrated Yap chromatin occupancy within promoters of myofibroblast-associated genes. In addition, Yap was found to predominantly occupy enhancer-enhancer loops and enhancer-promoter loops. Gene ontology analysis of these promoters, which were also detected by RNA-seq analysis, was enriched for pathways associated with inflammation and for activation of the proto-oncogene, Myc.

To elucidate the effect of *Lats1/2* depletion in CFs in the setting of disease, mice were subjected to myocardial infarction, which resulted in prolonged CF proliferation and altered scar formation, leading to lethality in all *Lats1/2* CKO mice within 3 wk. Massons' trichrome staining of the ischemic area revealed less dense, noncompacted collagen content in the *Lats1/2* CKO, suggesting that Hippo signaling was necessary for mature scar formation and the fully differentiated function of myofibroblasts.

The report of Xiao and colleagues elegantly shows how Hippo signaling is an integrated component of CF differentiation and activation in the adult mouse heart in normal homeostasis and with injury (Fig. 1), which also suggests the importance of cell-cell interaction in the heart (myeloid, endothelial, and cardiomyocytes). Like all pioneering work, numerous interesting questions arise that will be important to pursue in the future. What is the full scope of physiologic signaling in the nondiseased heart that leads to Hippo activity to maintain Yap/Taz in their inactive state (Fig. 1)? What role does stretch sensing play within the fibroblasts in regulating Hippo signaling versus inflammatory signals through membrane-bound receptors? More mechanistic investigation of endogenous Hippo signaling in CFs after injury is needed, such as annotating the dynamic regulation of Yap/Taz nuclear localization during myocardial infarction injury and other diseases with chronic fibrosis. There are also data suggesting that Yap/Taz can operate in a Lats1/2-independent manner (Panciera et al. 2017) and that Lats1/2 can mediate cell signaling beyond restricting Yap/Taz (Furth and Aylon 2017), which future studies of CF should address. Finally, it will be important to consider the development of new therapeutic approaches and ways to temporally activate or inhibit Hippo signaling in CFs to treat longstanding and progressive cardiac fibrotic

disease states but without impacting the regenerative responsiveness in cardiomyocytes from this same signaling pathway.

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