Beyond the Cardiomyocyte

Consideration of HIPPO Pathway Cell-Type Specificity

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Increasing interest in the HIPPO signaling pathway has stemmed largely from its ability to modulate cardioprotection and heart regeneration after injury, responses originating from manipulation within the cardiomyocyte. This viewpoint discusses the need for definitive understanding of HIPPO pathway function in cardiac nonmyocytes and highlights the necessity of considering cell-type specificity for effective therapeutic targeting of the HIPPO pathway in cardiovascular disease.

The HIPPO pathway is a highly conserved kinase cascade that determines organ size by regulating cell survival, proliferation, and differentiation. On activation, the core components of the pathway, MST (mammalian sterile 20-like kinase) 1/2, SAV1 (salvador 1), LATS (large tumor suppressor kinase) 1/2, and MOB1 (Mps one binder kinase activator-like 1), serve to phosphorylate and repress activity of the transcriptional cofactors YAP (Yes-associated protein) and TAZ (transcriptional coactivator with a PDZ-binding domain). When HIPPO is inhibited, YAP/TAZ cytosolic retention is relinquished, allowing for nuclear localization and association with multiple transcription factors, the most established being the TEAD (TEA domain) family (Figure 1). Investigation of the HIPPO pathway in the heart has garnered increased attention during the past decade and justifiably so. HIPPO is fundamental in maintaining adult heart homeostasis and modulating responses to injury. To date, the majority of studies investigating HIPPO signaling in the heart have focused on the cardiomyocyte. In response to myocardial infarction or pressure overload stress, the core HIPPO kinase MST1 is activated in cardiomyocytes. MST1 signals through canonical (to restrain YAP) and noncanonical (to antagonize BCL-XL [B-cell lymphoma-extra large] and Beclin1) mechanisms resulting in the inhibition of cardiomyocyte proliferation and autophagy and the enhancement of cell death.[1–3] Conversely, HIPPO pathway inhibition limits injury and promotes tissue regeneration through YAP-dependent increases in cardiomyocyte proliferation and survival.[4] As a result, interventions that activate cardiomyocyte YAP have become attractive and shown therapeutic potential.[5]

In contrast to the cardiomyocyte, HIPPO-YAP function in other cell types known to impact heart injury remains much less understood. It is clear that nonmyocytes outnumber cardiomyocytes in the heart and play essential roles in cardiac growth, homeostasis, and disease. Therefore, a more complete understanding of HIPPO-YAP function in nonmyocytes is imperative to furthering our comprehension, and improving the treatment, of cardiovascular disease.

HIPPO Pathway Function in Immune Cells

After stress, such as myocardial infarction, a sterile inflammatory response is rapidly activated in the myocardium. This is necessary to clear debris and promote wound healing after injury. However, excessive inflammation can augment matrix degradation, cause greater cardiomyocyte loss, increase fibrosis, and worsen heart function. Therefore, a balanced response is critical to provide maximum cardioprotection and optimal wound healing.

The HIPPO pathway can modulate both adaptive and innate immune responses. MST1 influences T cell survival, adhesion, chemotaxis, and proliferation—all important determinants of the immune response.[6] Recent work demonstrated that TAZ regulates T cell differentiation. TAZ promoted the development of T_H17 cells—a proinflammatory subtype that contributes to autoimmunity, while attenuating Treg cell production.[7] TAZ directly bound to RORγt (retinoic acid-related orphan receptor gamma t) to promote the T_H17 subset and potentiated autoimmune disease, implicating HIPPO pathway as a negative regulator of adaptive immune responses. In contrast, data from 2 recent studies suggest that activation of HIPPO signaling, and inhibition of YAP, is necessary for proper innate antiviral immunity. In macrophages, YAP directly interacted with IRF3 (interferon regulatory factor 3) to prevent IRF3 dimerization and nuclear translocation, thereby attenuating antiviral defense.[8] Additionally, YAP bound to TBK1 (TANK-binding kinase 1), preventing its association with signaling adapters and impeding IRF3 activation.[9] Taken together, these findings suggest that HIPPO-YAP/TAZ signaling may have opposing effects depending on immune cell subtype. Importantly, because HIPPO signaling impacts adaptive and innate immune cell function, it is likely to modulate both pathogen-triggered responses, for example, myocarditis, as well as sterile inflammation resulting from injury, for example, myocardial infarction. To what extent HIPPO function in immune cells impacts heart injury and healing remains unknown.

HIPPO Pathway in Vascular Cells

Endothelial and smooth muscle cells are critical components of the vasculature in the heart, and a functional role for HIPPO-YAP/TAZ has been demonstrated in both. Endothelial cell

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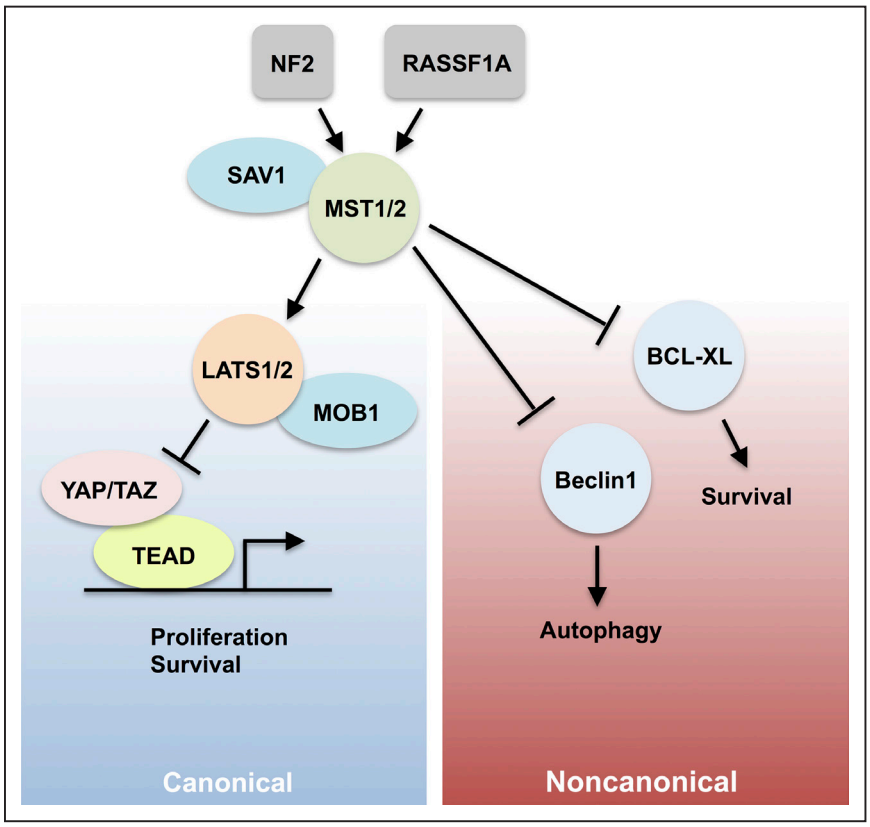

Figure 1. A simplified representation of the HIPPO pathway. On pathway activation, the core kinase MST (mammalian sterile 20-like kinase) 1/2 facilitates canonical signaling through phosphorylation and activation of LATS (large tumor suppressor kinase) 1/2 and the subsequent phosphorylation and inhibition of the transcriptional cofactors, YAP and TAZ. MST1 can also directly phosphorylate BCL-XL, leading to apoptosis, and Beclin1, which attenuates autophagy in cardiomyocytes. MOB1 indicates Mps one binder kinase activator-like 1; NF2, neurofibromin 2; and SAV1, salvador 1.

behavior is fundamentally influenced by blood flow-induced sheer stress. Laminar flow prevents leukocyte adhesion, inflammation, and atherosclerotic plaque formation, whereas sites of disturbed flow promote inflammation and are atheroprone. YAP/TAZ is activated in endothelial cells by disturbed flow and stimulates proliferation, inflammation, and promotes lesion formation.[10] Inhibition of YAP/TAZ in endothelial cells was shown to protect against the development of atherosclerosis and suggests that activation of HIPPO may be advantageous. However, endothelial YAP/TAZ has also been shown to promote vascular barrier formation and MYC-dependent proliferation to mediate angiogenesis in the developing nervous system.[11] Whether YAP/TAZ also promotes angiogenic responses after injury to the adult heart remains unknown. If so, there may be therapeutic potential in targeting YAP/TAZ activation (or HIPPO inhibition) to stimulate vessel formation during ischemia to enhance cardiomyocyte survival. Importantly though, any predicted benefit must be weighed against the potential for atherosclerotic progression. It has also been shown that thromboxane A2—a mediator of platelet aggregation—causes the repression of HIPPO, and the subsequent activation of YAP/TAZ, in vascular smooth muscle cells.[12] Inhibition of YAP/TAZ prevented proliferation and migration. Therefore, enhancing YAP/TAZ activation in this context could lead to unintended outcomes, for example, promoting restenosis, further increasing the complexity of HIPPO-YAP/TAZ signaling in the vasculature.

HIPPO Signaling in Fibroblasts

Recent work beautifully demonstrated that deficiency in HIPPO signaling (LATS1/2 kinase inhibition) causes the arrest of cardiac fibroblast differentiation during heart development—a response that is mediated by YAP/TAZ activation.[13] These findings indicate that, at least in the embryonic heart, aberrant YAP/TAZ function is sufficient to restrain cardiac fibroblast maturation from epicardial precursors, thereby disrupting proper extracellular matrix composition and subsequent coronary vasculature formation. In the setting of idiopathic pulmonary fibrosis, where tissue stiffness is augmented, YAP/TAZ was activated in lung fibroblasts and drove proliferation, profibrotic gene expression, and pathological matrix remodeling.[14] Importantly, little is known about the function of cardiac fibroblast HIPPO-YAP/TAZ in the adult heart. Although the latter study is not cardiac, it is possible that YAP/TAZ promotes a similar feed-forward fibrotic cycle to remodel the myocardium in response to injury.

Intercellular communication integrates signaling to modulate heart responses through autocrine and paracrine mechanisms. We previously examined the function of RASSF1A (Ras association domain family 1A)—a HIPPO pathway activator—in cardiomyocytes and cardiac fibroblasts in the context of pressure overload-induced hypertrophy and heart failure.[15] Interestingly, RASSF1A promoted HIPPO signaling in both cell types, but pathway engagement showed opposing outcomes for heart pathology and function. Systemic, but not cardiomyocyte-restricted, deletion of RASSF1A resulted in augmented cardiac fibrosis, and inhibition of RASSF1A-MST1 in cardiac fibroblasts increased proliferation, NF-κB (nuclear factor kappa B) activation, and TNFα (tumor necrosis factor alpha) secretion. Neutralization of TNFα in RASSF1A knockout mice was sufficient to prevent the hypertrophic and fibrotic phenotype, thereby demonstrating an important paracrine function downstream of HIPPO signaling that originates in the cardiac fibroblast. Although these

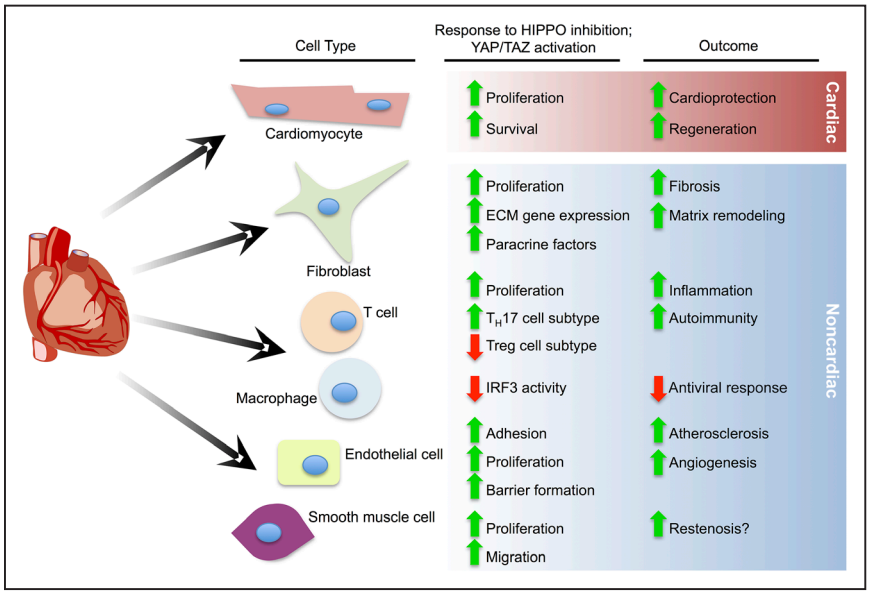

Figure 2. An abbreviated summary of phenotypes resulting from HIPPO inactivation or YAP/TAZ activation in cell types relevant to the adult heart. Generally, YAP/TAZ is restrained by basal HIPPO activity under physiological conditions. Further HIPPO activation can elicit opposite phenotypes of those noted, although this has not been validated in all cell types. To date, the majority of studies that focused on nonmyocyte HIPPO-YAP signaling were performed in noncardiac systems.

studies offer a glimpse into HIPPO-YAP fibroblast function in the adult heart, additional studies that leverage fibroblast-selective genetic manipulation in vivo are necessary to expand our understanding of cardiac fibrosis.

Concluding Remarks

The heart is a sophisticated organ consisting of multiple cell types that work in concert to move blood against resistance. In looking forward, it will be imperative to remain cognizant that these cell types fundamentally influence one another to maintain heart homeostasis and respond to challenges. Although much has been learned about HIPPO-YAP signaling in the cardiomyocyte, and efforts to manipulate YAP activation have shown promise, optimal interventions will likely require cell-type specificity because YAP activation in nonmyocytes may lead to adverse outcomes and negate cardiomyocyte benefit (Figure 2). Therefore, the ability to selectively modulate HIPPO-YAP activity by cell type may offer unique opportunities for future therapeutic development.

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