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EDITORIAL COMMENT

Drugging the Hippo (Pathway)

A Strategy to Stimulate Cardiac Regeneration?*

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Cardiac myocyte regeneration occurs in lower organisms such as zebrafish and newts after injury, but this capacity is lost in the adult mammalian myocardium when injurious stimuli such as myocardial infarction (MI) provoke cardiac myocyte loss, with disastrous clinical consequences. Accordingly, there has been tremendous interest in regenerating functional myocardium by cell therapy and/or strategies to stimulate cell division to replace dead cardiac myocytes. Clinical trials attest to the modest efficacy of exogenously delivered progenitor cells in improving cardiac function in humans with cardiomyopathy and heart failure; however, emerging consensus points to paracrine mechanisms with rapid loss of the delivered cells within hours after administration, precluding a sustainable therapeutic effect (1). A major breakthrough toward this goal has been the observation that existing mammalian cardiac myocytes divide at a low rate of 0.5% to 2.0% per year in humans (2) and rodents (3) throughout life. However, cardiac myocyte division is only modestly stimulated after injury (as in the border zone of an MI [3]), and there is no discernible contribution from resident or recruited progenitor cells to cardiac myocyte regeneration, underscoring the insufficiency of endogenous mechanisms for replacement of functional myocardium (reviewed elsewhere [1]).

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In contrast to the adult mammalian heart, in 1-day-old mouse pups, a robust regenerative response is seen in the immediate post-natal period driven by cell division in existing cardiac myocytes to restore normal myocardial architecture after surgical apical resection and ischemia-reperfusion injury (4). This regenerative potential is lost as early as 7 days of age in mice despite persistence of a small population of proliferating cardiac myocytes in the adult myocardium. Accordingly, targeting the signaling pathways that regulate cardiac myocyte proliferation after the early post-natal period has been an area of intense focus. In this issue of *JACC: Basic to Translational Science*, Hara et al. (5) perform complementary chemical screens to develop a pharmacological strategy to target the Hippo signaling pathway, which has been identified as a negative regulator of cardiac myocyte proliferation, in this setting (6).

The Hippo signaling pathway was discovered in *Drosophila* as a key regulator of organ size and plays critical roles in mammalian cardiac development, as well as cardiac myocyte differentiation and proliferation (reviewed by Wang et al. [7]). The mammalian Hippo signaling machinery comprises the serine-threonine kinases MST1 and MST2, which complex with the scaffold protein, Salvador 1, to phosphorylate and activate LATS1 and LATS2, another pair of serine-threonine kinases. The LATS kinases phosphorylate the transcriptional co-activator pair of YAP (Yes-associated protein) and TAZ (transcriptional co-activator with PDZ-binding motif) proteins to retain them in the cytosol and target for degradation, whereby active Hippo signaling tonically inhibits their transcriptional activity. Inhibition of the

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upstream Hippo signaling relieves the repression, resulting in nuclear translocation of YAP-TAZ and interaction with the TEA domain transcription factor (TEAD) to activate transcription of genes that stimulate cardiac myocyte proliferation and survival. The authors used clever screening approaches to identify small molecules that activate YAP-TEAD–mediated transcription, as well as a biological assay to identify TAZ activators that also activate TEAD-induced transcription, lending a high degree of specificity to their search (5). Focusing on readouts for deoxyribonucleic acid synthesis, karyokinesis, and cytokinesis, they performed careful chemical modifications to optimize the structure-activity relationship of a compound (TT-10) that promotes YAP-TAZ-TEAD–induced transcription by inducing nuclear translocation of YAP and stimulates cardiac myocyte division. Indeed, activation of YAP in cardiac myocytes in post-natal life is sufficient to stimulate cardiac myocyte proliferation, with induction of cell cycle genes; this action requires intact YAP-TEAD interaction (7).

Remarkably, TT-10 also activates β-catenin signaling by inhibiting phosphorylation of its upstream inhibitor, the glycogen synthase kinase 3 β isoform, which phosphorylates and targets β-catenin for degradation. Indeed, previous studies provide evidence for Hippo signaling cross-talk with the Wnt-β-catenin pathway to regulate cardiac progenitor proliferation (7). Upon inhibition of Hippo signaling, the YAP-TAZ coactivators form a complex with the β-catenin–T-cell factor/lymphoid enhancer factor transcription factor complex on promoters of genes that regulate cellular proliferation; β-catenin signaling is necessary for stimulation of cardiac myocyte division in this setting (8).

To translate these biological activities toward the goal of cardiac myocyte regeneration in a clinically meaningful fashion, the authors induced MI in mice followed by administration of a single dose of TT-10 (5). They observed a reduction in infarct size and attenuation of adverse post-MI ventricular remodeling. Remarkably, lineage-tracing experiments suggest TT-10 treatment stimulated clonal expansion of cardiac myocytes with nuclear YAP expression in the infarct border zone, with no evidence of discernible toxicity based on an extensive evaluation. These data confirm the observed upregulation of endogenous YAP1 protein and its nuclear localization in the infarct border zone in previous studies (9,10), and they provide a rationale for enhancing this endogenous regenerative response to titrate it to the clinical need. Indeed, studies have shown that further activation of YAP-TAZ signaling by inducible ablation of Salvador 1 three weeks after MI was sufficient to stimulate cardiomyocyte proliferation in the border zone (9) with rescue of post-MI cardiomyopathy. Conversely, haploinsufficiency of YAP1 in cardiac myocytes impaired the proliferative response in the border zone and accelerated apoptosis in cardiac myocytes, resulting in worsening of post-MI ventricular remodeling (10). An important challenge is whether the regenerated cardiac myocytes mature, and electrically and functionally integrate in the myocardial syncytium (1). Data presented by Hara et al. and others (9,10) suggest that stimulating this endogenous YAP-TAZ-TEAD activation response may achieve this goal commensurately with reduced fibrosis and adequate vascularization in the MI border zone.

Unbiased transcriptional profiling of TT-10–treated cardiac myocytes also revealed activation of NRF-2–mediated transcription, resulting in up-regulation of antioxidant responses with antiapoptotic effects (5). Indeed, the data indicate that inhibition of cardiac myocyte death is likely to be a significant contributor to the observed beneficial effects on the myocardium, post-MI. Notably, YAP signaling in unstressed adult myocardium has been predominantly ascribed a prosurvival role, as cardiac myocyte-specific ablation of YAP1 in the post-natal heart triggered widespread cardiomyocyte death due to apoptosis, resulting in cardiomyopathy (10). Definitive evidence for the contribution of myocardial salvage via inhibition of cardiomyocyte death vis-à-vis myocardial regeneration will require targeted loss-of-function approaches to target either mechanism, concomitant with TT-10 administration. Intriguingly, studies indicate that oxidative stress suppresses the endogenous proliferative activity in adult cardiac myocytes (4) via suppression of transcription factor Pitx2 activity, which is upregulated in Hippo-deficient hearts and is sufficient to drive cardiac myocyte proliferation (11). By tilting the scales away from death toward proliferation, this compound may also affect noncardiac myocytes, a premise that will need careful evaluation with contemporary genetic approaches to understand the mechanisms for observed beneficial effects.

Overall, this study by Hara et al. (5) presents a remarkable drug discovery effort to validate the YAP-TAZ-TEAD complex as a drug target for cardiac regeneration. Further pharmacokinetic and pharmacodynamic evaluations will be necessary in large animal models to understand whether sustainable benefits accrue without significant toxicity, before translation to humans. The underlying biology also dictates a need for caution with this approach as

inhibition of Hippo signaling can trigger oncogenic transformation (reviewed elsewhere [12]), and unbridled activation of YAP-TAZ-mediated transcription stimulates exuberant cardiac myocyte proliferation post-injury in mice (13). Indeed, threading this needle carefully could be the difference between losing this opportunity "in translation" or widespread application of a novel class of compounds that "drug the Hippo" to realize the dream of cardiac regeneration.

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