The Hippo/Yes-associated protein (Yap) pathway has recently received significant attention in the field of cardiac biology as it has come to light that it may hold great promise in the development of treatments for heart disease. First discovered in Drosophila, the Hippo/Yap pathway controls organ size during development in a highly conserved manner. The mammalian core Hippo kinase cascade includes Ste20-like kinases (Mst1/2), scaffolding proteins Sav and Mob, and large tumor suppressor kinases (Lats1/2). Upon phosphorylation by Mst kinases, Lats kinases target Yap for phosphorylation, leading to its sequestration in the cytoplasm. Conversely, when the Hippo pathway is inactive, Yap can translocate to the nucleus where it acts as a transcriptional coactivator for the TEAD family of transcription factors. In this role, Yap activates a gene expression program associated with cell proliferation and survival.

The Hippo pathway has been shown to be a critical regulator of heart growth and development. Moreover, several reports have shown that modulating activity of the Hippo/Yap pathway can improve the heart’s response to murine models of cardiovascular disease and injury. The Olson and Martin groups showed that inhibition of Hippo components or expression of a constitutively active form of Yap (YapS112A) are each able to extend the normal window of cardiomyocyte proliferation into the adult stage, leading to improved cardiac function after coronary ligation ischemic injury.9 In moderation, this observed increase in Yap activity leads to functional regeneration and improved cardiac function after coronary ligation ischemic injury. However, long-term Yap activity activates a fetal gene expression program and causes cardiac dysfunction. Modulating the Hippo/Yap pathway to treat cardiac injury and disease will require further understanding of the mechanism by which Yap mediates its pro-growth, pro-survival gene expression program. The studies in the current issue of Circulation Research by Yang et al10 contribute to our understanding of Hippo signaling and identify an miRNA-mediated pathway downstream of Yap in the heart (Figure). These studies provide new insight into how Yap functions in the adult heart and, as with all good science, answer some existing questions, as well as provoke new questions.

Yang et al identified miR-206 in a microarray screen for upregulated miRNAs in neonatal rat cardiomyocytes overexpressing Yap. Previously, miR-206 had been shown to play an important role in skeletal muscle hypertrophy.11 Yang et al performed a series of in vitro experiments that led them to conclude that the Hippo/Yap pathway regulates cardiomyocyte hypertrophy and survival through the actions of miR-206. Adenoviral-mediated overexpression of Yap in neonatal rat cardiomyocytes led to increased miR-206 expression, whereas overexpression of Mst1, a critical kinase in the Hippo pathway, led to decreased miR-206 expression. To draw a functional connection between Yap and miR-206, the authors show that adenoviral-mediated overexpression of miR-206 or Yap in neonatal rat cardiomyocytes produced similar changes in cardiomyocyte hypertrophy and cell viability after treatment with the apoptosis inducer chelerythrine. Combined loss of miR-206 and overexpression of Yap in vitro abrogated the phenotypic changes associated with overexpression of Yap.

The authors then generated transgenic cardiac-specific gain- and loss-of-function mouse models to study the role of miR-206 in vivo. These studies showed that overexpression of miR-206 leads to cardiac hypertrophy, increasing in heart weight/body weight ratio, left ventricular weight/body weight ratio, cross-sectional area of cardiomyocytes, and expression of the hypertrophy marker atrial natriuretic factor (ANF). Interestingly, no change in expression of proliferation marker Ki67 was observed. Furthermore, normal cardiac function was noted as measured by left ventricular ejection fraction and lung weight/body weight ratio. Decreased miR-206 activity using a transgenic miRNA sponge construct containing 2 repeats of antisense miR-206 driven by the α-MHC (myosin heavy chain) promoter reduced the cardiomyocyte hypertrophy observed upon overexpression of miR-206. The authors also show that coronary ligation injury in these miR-206 loss-of-function transgenic animals results in a worsening of the infarct size. This was in contrast to the gain-of-function miR-206 overexpressing mice, which displayed a better outcome after ischemic injury as noted by reduced infarct size and better cardiac function. Finally, using locked nucleic acids in an additional loss-of-function approach, wild-type mice were treated with locked nucleic acid–anti-206 and then subjected...
to transverse aortic constriction. Transverse aortic constriction led to an increase in left ventricular weight/tibia length ratio, cardiomyocyte cross-sectional area, and expression of ANF in control mice. Treatment with locked nucleic acid–anti-206 reduced these responses. From these experiments, the authors conclude that miR-206 serves to protect the heart against injury and progression to heart failure.

Bioinformatic analysis identified Foxp1 as a potential target of miR-206, with 4 predicted miR-206 binding sites located in its 3′UTR. Adenoviral-mediated expression of miR-206 in neonatal cardiomyocytes led to decreased luciferase reporter activity using a portion of the Foxp1 3′UTR located in its 3′UTR. Mutation of these binding sites abrogated the ability of miR-206 to repress this reporter. This led the authors to conclude that Foxp1 is a direct target of miR-206 in cardiomyocytes. Furthermore, neonatal cardiomyocytes subjected to adenoviral-mediated overexpression of miR-206 or Yap, as well as transgenic hearts overexpressing miR-206, displayed decreased Foxp1 protein levels. In contrast, transgenic miR-206 loss-of-function hearts expressed increased levels of Foxp1, which were normalized in the presence of the transgenic miR-206 overexpressing allele. In vitro, adenoviral-mediated overexpression of Foxp1 also normalized the increase in cardiomyocyte size that resulted from miR-206 or Yap overexpression alone. However, with regards to miR-206’s and Yap’s abilities to protect against chelerythrine-induced cardiomyocyte apoptosis, Foxp1 overexpression completely inhibited the effect of miR-206 but only partially inhibited that of Yap. This data suggest that miR-206 promotes cardiomyocyte hypertrophy and survival by downregulating Foxp1, whereas Yap may have additional mechanisms by which it promotes survival.

Although this work intriguingly outlines an miRNA-mediated path that mediates effects of Yap, it does so with a focus on the role of Yap in promoting cardiomyocyte hypertrophy. In contrast, there is a growing body of evidence that the Hippo/Yap pathway controls heart size through regulation of cardiomyocyte proliferation during development and can promote cardiomyocyte proliferation in the adult heart. In vivo expression of constitutively active YapS112A in the embryonic heart leads to increased cardiomyocyte proliferation along with decreased cardiomyocyte size. Accordingly, conditional knockout of Hippo components Sav or Lats1/2 leads to increased cardiomyocyte proliferation with no change in cardiomyocyte size in the developing heart. During the neonatal period of heart growth, when cardiomyocytes switch from a proliferative to a hypertrophic mode of growth, physiological expression of Yap decreases. Furthermore, transgenic loss of Yap does not impair physiological hypertrophy of cardiomyocytes during the postnatal period.

Interpreting the effects of modulating the Hippo/Yap pathway is more complicated in experimental models of heart disease and injury. Conditional knockout of Sav in the heart results in increased cardiomyocyte proliferation and reduced apoptosis after ischemic injury at both the neonatal and adult stages. Ischemic injury in neonatal and adult mice expressing YapS112A leads to increased cardiomyocyte proliferation and survival, resulting in decreased fibrosis and improved cardiac function. However, this increase in proliferation, especially at the adult stage, is modest. Although increased cell survival and proliferation seem to be primary contributors, it is conceivable that hypertrophy also contributes to the observed regeneration. In fact, the current authors have previously shown that adenoviral-mediated overexpression of Yap leads to increased hypertrophy, as well as cell survival and proliferation in vitro. They have also shown that transgenic expression of a dominant negative form of Lats2 leads to increased cardiac hypertrophy both at baseline and in response to pressure overload in cardiomyocytes. Interestingly, a recent study reported that tissue samples from patients with hypertrophic cardiomyopathy displayed increased Yap expression and decreased Yap phosphorylation in conjunction with increased Mst1 expression. This study also reported that cardiac-specific transgenic expression of human Yap in mice led to a phenotype resembling hypertrophic cardiomyopathy, and Yap expression in cultured cardiomyocytes led to increased cell size. Together, these studies highlight that
much more research is needed to understand the role of Hippo/Yap in the adult heart and in response to cardiac injury.

This work also helps to address longstanding questions regarding the role of hypertrophy in heart disease. The current study showed that miR-206 does not affect cardiac function at baseline but improves the heart’s response to injury. This led to the authors’ conclusion that Yap-induced increase in miR-206 expression mediates physiological hypertrophy that serves as a compensatory response to protect the heart against stressors. Cardiac hypertrophy is a complex response to both injury and chronic stress. Although hypertrophy is necessary to increase output in the absence of productive cardiomyocyte regeneration, it can lead to cardiac failure in the long term. Part of this dichotomy is caused by the activation of abnormal gene expression programs associated with the fetal heart. These gene expression programs can, on the one hand, provide potent pro-survival signals and, on the other hand, impair proper contractility and metabolism. Thus, modulating pathways, such as Hippo/Yap, in attempt to control cardiac hypertrophy or promote cardiomyocyte regeneration will have to be done cautiously. In particular, inhibition or activation of Hippo/Yap will have to be controlled in a tight temporal manner given the deleterious effects of long-term tonic Yap activity in the adult heart.

Foxp1 has previously been established to repress genes associated with a fetal gene expression program, including Myh7, Bnp, and Anf.15 Thus, it seems reasonable to consider the possibility that overexpression of miR-206, which could repress Foxp1, could reactivate a fetal gene expression program. This could lead to deleterious results if not carefully controlled. Future studies will be important to explore the consequences of long-term gain or loss of miR-206 activity.

The longstanding dilemma of how to define the responses of the heart to stress and injury that are beneficial verses those that are maladaptive and ultimately contribute to heart failure will undoubtedly benefit from the current report, as well as further research. This article contributes to this effort by uncovering a new Yap-mediated signaling pathway that may play an important role in the response of the adult heart to injury.

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References


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