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Invited Review

An emerging role for Hippo-YAP signaling in cardiovascular development

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Abstract

The Hippo signaling pathway was originally discovered in *Drosophila* and shown to be critical for organ size control and tumorigenesis. This pathway consists of a cascade of kinases and several adaptors that lead to the phosphorylation and inhibition, through nuclear exclusion, of the transcriptional cofactor Yorkie in *Drosophila* or YAP (yes associated protein) in mammals. Recent studies demonstrate that cardiac-specific deletion of the Hippo pathway kinase Mst (STE20-like protein kinases) co-activator WW45 (WW domain-containing adaptor 45), Mst1, Mst2, or Lats2 (large tumor suppressor homologue 2) in mice result in over-grown hearts with elevated cardiomyocyte proliferation. Consistent with these observations, over-expression of YAP in the mouse embryonic heart increases heart size and promotes cardiac regeneration and contractility after myocardial infarction by inducing cardiomyocyte proliferation, whereas deletion of YAP in the mouse heart impedes cardiomyocyte proliferation, causing myocardial hypoplasia and embryonic or premature lethality. YAP has also been shown to play an important role in the vascular system. Specific-deletion of YAP from vascular smooth muscle cells in mice results in aberrant development of large arteries with a hypoplastic arterial wall phenotype. Hippo-YAP signaling cross-talks with other signaling pathways such as IGF (insulin-like growth factor) and Wnt signaling to promote heart growth by increasing expression of cell cycle genes. The purpose of this review is to summarize these recent findings and discuss potential diagnostic or therapeutic strategies in cardiovascular system based on manipulating the Hippo-YAP signaling.

Keywords: Hippo, YAP, cardiac development, vascular smooth muscle, proliferation

INTRODUCTION

The heart is the first organ to form during mammalian embryogenesis. Together with blood vessels, these circulatory organs provide nutrient perfusion necessary for further organogenesis. In the mouse, heart formation starts from cardiogenic precursor cells located in the cardiac crescent, to form the linear heart tube at about embryonic day (E) 8.0. The heart tube then initiates rightward looping and chamber specification, followed by septation, and valvulogenesis[1]. The mouse embryo starts to form blood vessels through vasculogenesis at E7.5. A coordination of multiple cell types including endothelial and smooth muscle cells (SMCs) is required for a proper pattering of the vasculature. Soon after establishment of a capillary network by endothelial cells, vascular SMC progenitors begin to invest the vessel wall through migration and proliferation, meanwhile undergoing differentiation to acquire a unique array of smooth muscle-contractile proteins[2]. Understanding the transcriptional networks that control cardiovascular development and their underlying control

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mechanisms is not only critical for identifying genes whose defects cause congenital cardiovascular disorders in humans but also provides a molecular basis for diagnostic and therapeutic strategies.

The Hippo signaling pathway was originally discovered in *Drosophila* and subsequent genetic and biochemical studies revealed that the pathway components and functions are evolutionarily conserved through humans[3]. This pathway includes a cascade of kinases and several adaptors that lead to the phosphorylation and inhibition of the transcriptional cofactor Yorkie by preventing its nuclear translocation. The core components of the pathway consist of the Ste20-like kinases Hippo and its regulatory protein Salvador, the downstream kinase Warts and its regulatory protein Mats. The Hippo-Salvador complex phosphorylates the Warts-Mats complex, which, in turn, phosphorylates the oncoprotein Yorkie, which inactivates Yorkie by retaining it in the cytoplasm. In the absence of Hippo signaling, unphosphorylated, active Yorkie translocates into the nucleus and functions as a co-activator with the TEAD (TEA domain) family transcription factor Scalloped, to induce genes that promote cell growth and oncogenic transformation[4,5]. Due to the high evolutionary conversation, Hippo, Salvador, Warts, Mats, Yorkie and Scalloped in *Drosophila* are structurally and functionally homologous to mammalian MST1/2, WW45, LATS1/2, Mob1 (Mps one binder), YAP and TEAD, respectively. YAP has been shown to be a critical determinant of organ size[6]. YAP is also considered as a potent oncoprotein by promoting cell growth and inhibiting cell apoptosis[7].

Recently, several studies showed that the Hippo-YAP pathway plays a critical role in mouse cardiovascular development. The focus of this review is to summarize recent progress in understanding the molecular basis underlying Hippo-YAP signaling in cardiovascular development. Other functions of Hippo-YAP signaling have been discussed in several recent excellent review articles[8–10].

ROLE OF HIPPO PATHWAY COMPONENTS IN MOUSE CARDIAC DEVELOPMENT

Extensive genetic studies have shown Hippo pathway components are critical for mouse embryogenesis and tumorigenesis in mice. Salvador is a key component of the Hippo pathway that restricts cell proliferation and promotes apoptosis[11,12]. Conventional ablation of the Salvador homologue WW45 in mice leads to embryonic lethality with hyper-proliferative epithelial cells of the skin and intestine accompanied by immature differentiation[13]. Many organs in the WW45 mutant embryos display hyperplasia. However, this study did not examine the possibility that embryonic lethality is attributable to a cardiac phenotype as it would be a little surprising if the skin or intestinal phenotypes resulted in embryonic mortality. Although Mst1/2 are tumor suppressors due to their ability to restrict cell proliferation[14], unexpectedly, mice lacking both Mst1 and 2 do not exhibit a hyper-proliferative phenotype. Rather, the *Mst1/2* double knockout mice exhibit severe growth retardation, failed placental development, impaired yolk sac/embryo vascular patterning and primitive hematopoiesis and die at approximately E8.5[15]. The majority of mice with global deletion of the Mst1/2 downstream kinase Lats1 die by postnatal day 1 and surviving mice develop large soft tissue sarcomas, ovarian stromal cell tumors and are susceptible to carcinogenic treatments[16]. *Lats2* global knockout mice are embryonic lethal between E10.5–12.5 and display pleiotrophic developmental defects[17], suggesting unique functions of Lats2 in development. The severe phonotypes observed in the mice harboring genetic deletion of Hippo pathway components described above suggest Hippo signaling is critical for embryogenesis and tumorigenesis in mice.

To begin to examine the effects of the Hippo pathway more specifically during cardiac development a number of groups have generated cardiac-specific knockouts of the pathway components. The cardiac specific knockout of the Mst kinase co-activator WW45 (Salvador homologue in mammals) in mice resulted in over-grown hearts with elevated cardiomyocyte proliferation[18]. *WW45* cardiac-specific KO hearts display expansion of trabecular and subcompact ventricular myocardial layers and have thickened ventricular walls without a change in cardiomyocyte cell size. *Lats2* and *Mst1/2* cardiac-conditional KO hearts have similar myocardial expansion phenotypes[18]. Mechanistically, the study reported that the Hippo signaling interacts with the Wnt signaling effector β-catenin to regulate several cell cycle genes thereby promoting cardiomyocytes cell proliferation. However, how the Hippo signaling cross-talks to Wnt signaling in the heart is still elusive. Furthermore, the function of upstream components above Mst/WW45 in Hippo signaling in the cardiac and vascular development remains to be determined.

ROLE OF YAP IN MOUSE CARDIOVASCULAR DEVELOPMENT

YAP null mice die at E8.5 with defects in yolk sac vasculogenesis, chorioallantoic attachment, and embryonic axis elongation[19], precluding an analysis of heart development. Cardiac-specific inactivation of

YAP, early during development mediated by either Nkx2.5 Cre or Tnnt2 Cre, impedes cardiomyocyte proliferation, causing myocardial hypoplasia and embryonic lethality in mice[20,21]. By using α-MHC cre, which deletes YAP at later stages of heart development, *YAP* knockout mice complete embryonic development but mutant mice died prematurely starting around 11 weeks of age due to dilated cardiomyopathy resulted from thinning ventricular walls[22,23]. These *YAP* knockout mice also exhibit impaired neonatal heart regeneration, leading to a default fibrotic response and exacerbated myocardial infarction induced cardiac impairment[22,23]. Similar to the mice with loss-of-function of Hippo pathway kinases, over-expression of YAP in mouse embryonic heart increases heart size by promoting cardiomyocyte proliferation without significantly changing cardiomyocyte size[20,22]. The gain-of-function YAP phenotype in the mouse heart largely phenocopies the cardiac-specific knockout of Salvador, Mst1/2 and Lats2 in the mouse heart. However, the cardiac-specific *WW45* knockout mice display a ventricular septal defect[18], which is not observed in mice in which YAP overexpression is driven by either the cardiac-specific α-*MHC,* β-*MHC* or *Tnnt2* promoters. This discrepancy suggests that there must be other effectors of down-stream of Hippo signaling in the heart in addition to YAP, although YAP is the major effector that regulates cardiomyocyte proliferation. Consistent with the impaired regeneration seen in *YAP* knockout mice, over-expression of YAP in the mouse heart promotes cardiac regeneration and improves contractility after myocardial infarction[22]. These exciting findings suggest that therapeutic strategies to increase YAP expression or activate the existing YAP would be beneficial for treatment of myocardial infarctions. Together, these gain-of-function and loss-of-function assays unequivocally demonstrate that YAP is necessary for embryonic and neonatal heart growth. Mechanistically, all of reports described above unambiguously demonstrate that YAP functions in cardiomyocytes to promote proliferative gene programs. Although manipulation of YAP expression levels in the heart provides a potential strategy to repair heart injury by inducing cardiomyocyte proliferation, the role of upstream Hippo kinases in the cardiac regeneration warrants further investigation.

In addition to being important for cardiac development, we have recently shown that YAP also plays an important role in vascular development[24]. Previously we found that YAP plays a novel integrative role in smooth muscle phenotypic modulation by inhibiting smooth muscle-specific gene expression while promoting smooth muscle proliferation and migration *in vitro* and *in vivo*[25]. Similar to the ''synthetic'' phenotype that SMCs exhibit in response to arterial injury, SMCs are highly proliferative and migratory during vasculogenesis. Given the important role of YAP in regulating cell proliferation, we tested the importance of YAP in regulating SMC proliferation during vascular development. We found that cardiac and smooth muscle cell-specific deletion of YAP directed by SM22α-Cre resulted in perinatal lethality in mice due to profound cardiac defects. The cardiac/smooth muscle-specific *YAP* knockout mice also displayed severe vascular defects[24]. Unexpectedly, deletion of YAP in mouse vascular SMCs did not alter the expression of smooth muscle-contractile proteins or the proliferative genes that are involved in cardiomyocyte proliferation[18,20,21]. Rather, *YAP* knockout induced expression of a subset of cell cycle arrest genes including Gpr132[24]. Over-expression of Gpr132 attenuated SMC growth by arresting cell cycle in G0/G1 phase, suggesting deletion of YAP induced impairment of SMC proliferation is mediated, at least in part, by induction of Gpr132 expression. Our study not only suggests a crucial role of YAP in arterial development in mice, but also identifies a novel mechanism through which YAP promotes vascular SMC proliferation by repressing cell cycle arrest gene expression. Due to the embryonic and perinatal lethality of the YAP mutant mice directed by the *SM22α* promoter driven cre transgene, a smooth muscle-specific inducible KO model is required to further study role of YAP in adult smooth muscle cells. Whether deletion of YAP in adult SMCs can attenuate the vascular remodeling induced by vascular injury is currently under investigation in our group by using an inducible SM MHC promoter-driven cre mouse line[26].

FUTURE PERSPECTIVES

In a relative short period of time, several studies have highlighted the importance of the Hippo-YAP pathway in heart development and regeneration. It is quite likely that mutation of genes or disregulation of the Hippo-YAP pathway may be involved in pathogenesis of congenital heart diseases associated with abnormalities of myocardial growth in humans. These studies suggest that analysis of Hippo-YAP components may be a useful diagnostic strategy for identification of congenital cardiovascular disorders in humans. Moreover, the strong effects of deletion of Hippo kinases or overexpression of YAP on cardiomyoctye proliferation offers great promise of targeting this pathway as a therapeutic strategy in cardiac regenerative medicine. Simply, by activation of YAP or/and inactivation Hippo kinases in the heart, it may be possible to promote cardiomyocyte proliferation to repair the heart after injury and thereby limit the deleterious effects

of myocardial infarctions. However, YAP's ability to promote cell proliferation may be a ''double-edged sword'' for vascular SMCs because the increased proliferation of vascular SMCs is a major contributing event leading to the development of occlusive vascular diseases after arterial injury. YAP is significantly induced in rodent carotid artery injury models and deletion of YAP attenuates injury-induced neointima formation[25]. Therefore blocking the induction of YAP would be a potential therapeutic approach for ameliorating vascular occlusive diseases. These data suggest that any therapies aimed at targeting the Hippo-YAP pathway in the cardiovascular system will need to be spatially and temporally targeted carefully.

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