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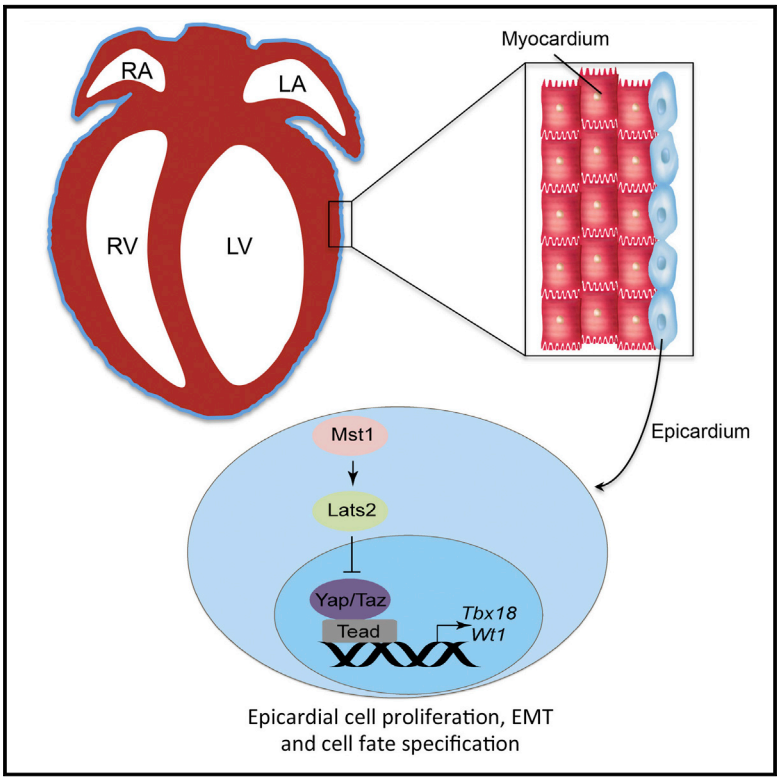
Report

Hippo Signaling Mediators Yap and Taz Are Required in the Epicardium for Coronary Vasculature Development

Graphical Abstract



Highlights

- Hippo signaling components are expressed in the developing proepicardium and epicardium
- Genetic deletion of Yap and Taz leads to coronary vasculature defects
- Yap and Taz regulate epicardial cell proliferation, EMT, and cell fate specification

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In Brief

Singh et al. show that Hippo signaling components are expressed in proepicardial and epicardial cells and are required for coronary vasculature development. Yap and Taz regulate epicardial cell proliferation, EMT, and cell fate specification, in part by regulating *Tbx18* and *Wt1* expression.

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Hippo Signaling Mediators Yap and Taz Are Required in the Epicardium for Coronary Vasculature Development

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SUMMARY

Formation of the coronary vasculature is a complex and precisely coordinated morphogenetic process that begins with the formation of epicardium. The epicardium gives rise to many components of the coronary vasculature, including fibroblasts, smooth muscle cells, and endothelium. Hippo signaling components have been implicated in cardiac development and regeneration. However, a role of Hippo signaling in the epicardium has not been explored. Employing a combination of genetic and pharmacological approaches, we demonstrate that inhibition of Hippo signaling mediators Yap and Taz leads to impaired epicardial epithelial-to-mesenchymal transition (EMT) and a reduction in epicardial cell proliferation and differentiation into coronary endothelial cells. We provide evidence that Yap and Taz control epicardial cell behavior, in part by regulating *Tbx18* and *Wt1* expression. Our findings show a role for Hippo signaling in epicardial cell proliferation, EMT, and cell fate specification during cardiac organogenesis.

INTRODUCTION

The coronary vasculature is required for supplying oxygenated blood to the cardiac muscle. Proper coronary blood circulation is essential for embryonic and adult cardiac tissue homeostasis. Defects associated with the coronary function leads to myocardial ischemia, infarction, and heart failure. Therefore, identifying molecules and signaling pathways regulating coronary vessels morphogenesis, remodeling, and maturation is essential in understanding the etiology of coronary diseases. The incidents of coronary anomalies have been reported in up to 1% of the general population (Angelini, 2002). During embryogenesis, cells from multiple sources, including the proepicardium and epicardium, contribute to the development of coronary vasculature (Chen et al., 2014; Red-Horse et al., 2010; Wu et al., 2012). The epicardium is a single layer of epithelial cells that covers the heart. It develops from the proepicardial organ (PEO), a transient structure that arises from the mesothelium of the septum transversum (Männer, 1993; Mikawa and Gourdie, 1996). The epicardium plays a significant role in heart development and gives rise to the majority of cells, including fibroblasts, smooth muscle cells, and endothelium, of the coronary vasculature (Männer, 1993; Mikawa and Gourdie, 1996; Singh and Epstein, 2012; Singh et al., 2011). Epicardium-deficient hearts exhibit impaired cardiac function due to a thin myocardium, suggesting that factors secreted from the epicardium are required not only for coronary vasculature development but also for the proliferation and differentiation of the underlying myocardial cells (Männer, 1993; Männer et al., 2005; Pennisi et al., 2003). The role of the epicardium in cardiac homeostasis was recently explored using an epicardial injury model. Developmental gene programs were re-activated following injury, which led to epicardial cell expansion and differentiation into cardiac fibroblasts and smooth muscle cells (Zhou et al., 2011). A better understanding of embryonic epicardial biology will help to understand the pathophysiology of coronary defects and it may suggest strategies to manipulate adult epicardial cells to facilitate myocardial regrowth and angiogenesis after cardiac injury.

The Hippo signaling is an evolutionary conserved pathway that control organ size by regulating cell proliferation, cell survival, and stem cell self renewal (Zhao et al., 2011). Hippo signaling has been implicated in cardiac development as well as in cardiac repair and regeneration after myocardial injury. Genetic deletion, with a cardiac-specific Cre-recombinase, of *Mst1/2*, *Lats2*, or *Salvador* (*Salv*) leads to an expansion of ventricular myocardium due to increased cardiomyocyte proliferation (Heallen et al., 2011). Global deletion of *Yap* results in embryonic lethality around embryonic day 8.5 (E8.5) due to defects in yolk sac vasculogenesis, chorioallantonic fusion, and body axis elongation (Morin-Kensicki et al., 2006). However, *Taz* knockout mice are viable through adulthood, although some