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## Hippo Activation in Arrhythmogenic Cardiomyopathy

Yong Hu, William T. Pu

Arrhythmogenic cardiomyopathy (AC) is an inherited heart muscle that affects  $\approx 1$  to 2 in 5000 individuals and accounts for 15% to 25% of cases of sudden cardiac death in patients <35 years.<sup>1</sup> The cardiomyopathy is characterized by risk for lethal ventricular arrhythmias, ventricular enlargement and dysfunction, and fibro-fatty replacement of cardiomyocytes. Curiously, the disease often disproportionately involves the right ventricle and hence also has been referred to as arrhythmogenic right ventricular cardiomyopathy.

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The molecular genetics of AC indicate that it is a disease of the desmosome, intercellular junctional complexes found in epithelium and in muscle tissue.<sup>2</sup> In heart, desmosomes are concentrated at the intercalated discs (IDs) that join the ends of cardiomyocytes. Breakthrough work by McKoy et al<sup>3</sup> identified a frameshift mutation in the desmosome protein plakoglobin (PG, also known as JUP) as the cause of Naxos disease, a rare, autosomal-recessive form of AC. Subsequently,  $\approx 60\%$  to 65% of more typical autosomal-dominant forms of AC were found to be because of mutations in genes encoding other components of the desmosome, including Plakophilin-2 (PKP2), Desmoglein-2 (DSG2), Desmocollin-2 (DSC2), and Desmoplakin (DSP).<sup>2</sup>

How does disruption of desmosomes lead to ventricular dysfunction, fibro-fatty replacement of myocytes, and a proarrhythmic substrate? Initial studies focused on the structural consequences of weakened intercellular adhesions and disruption of intercellular contacts (Figure). More recent studies also examined the effect that disruption of desmosomes has on intracellular signaling. A common feature of AC mutations is that they diminish PG localization to IDs.<sup>4</sup> Indeed, AC mutations cause PG to localize to the nucleus.<sup>5</sup> Intriguingly, PG is also known as  $\gamma$ -catenin and has 69% amino acid identity with its better known cousin  $\beta$ -catenin. In addition to participating in intercellular adhesion,  $\beta$ -catenin is also well known for its role as a transcriptional coactivator of the canonical Wnt signaling pathway. Wnt/ $\beta$ -catenin signaling is a key regulator of myogenesis versus adipogenesis. In AC, nuclear PG has been proposed to contribute to AC pathogenesis by suppressing canonical Wnt signaling and thereby enhancing adipogenesis driven by PPAR $\gamma$  and C/EBP $\alpha$  (Figure).<sup>5-7</sup>

In this issue of *Circulation Research*, Chen et al<sup>8</sup> link desmosome disruption in AC to perturbation of another key intracellular signaling pathway, the Hippo/YAP pathway (Figure). This ancient pathway is central to regulation of organ growth and cellular proliferation and has been linked recently to control of cardiomyocyte proliferation and heart size.<sup>9-11</sup> The core of the pathway consists of the Hippo kinase cascade, comprising mammalian MST1/2 (orthologs of *Drosophila* Hippo, the pathway's namesake) and LATS1/2. MST1/2 phosphorylates and activates LATS1/2, which in turn phosphorylates YAP or WWTR1 (also known as TAZ), transcriptional coactivators that mediate nuclear responses to Hippo signaling. Phosphorylation inhibits YAP and TAZ transcriptional activity by promoting their nuclear export and cytoplasmic sequestration. Consistent with their role in orchestrating cell crowd control, the activities of Hippo kinases and of YAP/TAZ are tightly regulated by cell-cell interactions. Both MST and LATS localize to the submembrane region, where their activities are regulated by interaction with NF2.<sup>12</sup> YAP also localizes to this submembrane region by interacting with intercellular adhesion complex proteins  $\alpha$ -catenin<sup>13</sup> and angiominin.<sup>14</sup> These interactions, stimulated by Hippo kinase phosphorylation of YAP, are additional mechanisms by which cell-cell contacts regulate YAP transcriptional activity.

The study of Chen et al<sup>8</sup> examined myocardial samples from both AC patients and mouse AC models. AC caused remodeling of IDs, with reduced levels of desmosome proteins including PG, as well as the gap junction Connexin 43 (GJA1) and N-cadherin (CDH2).<sup>8</sup> The mechanism(s) underlying this ID remodeling has not been determined and might include impaired ID assembly, reduced ID stability/increased turnover, and abnormal regulation of ID gene expression by nuclear plakoglobin.<sup>15</sup> ID remodeling reduced localization of activated PKC $\alpha$  to these structures. PKC $\alpha$  normally phosphorylates and inactivates NF2. Consequently, there was greater activation of NF2 and Hippo kinases, resulting in increased localization of phosphorylated YAP to cell junctions. These findings were recapitulated in cultured HL-1 cardiomyocyte-like cells by PKP2 knockdown. In this system, RNA-seq demonstrated a transcriptional signature consistent with increased Hippo kinase activity, and this was confirmed by luciferase reporter assay showing decreased YAP transcriptional activity. These data identify a new mechanism by which cell-cell contacts regulate Hippo/YAP signaling and are potentially relevant to other desmosome-containing tissues in addition to heart muscle.

Interestingly, the Hippo/YAP pathway is intertwined with Wnt/ $\beta$ -catenin signaling. For example, activated Hippo phosphorylates TAZ, promoting its localization to the submembrane region, where it binds and inhibits disheveled, an essential transducer of Wnt signaling.<sup>16</sup> YAP has been shown to bind  $\beta$ -catenin and augment Wnt/ $\beta$ -catenin-driven transcription.<sup>11</sup> YAP- $\beta$ -catenin interaction has also been shown to

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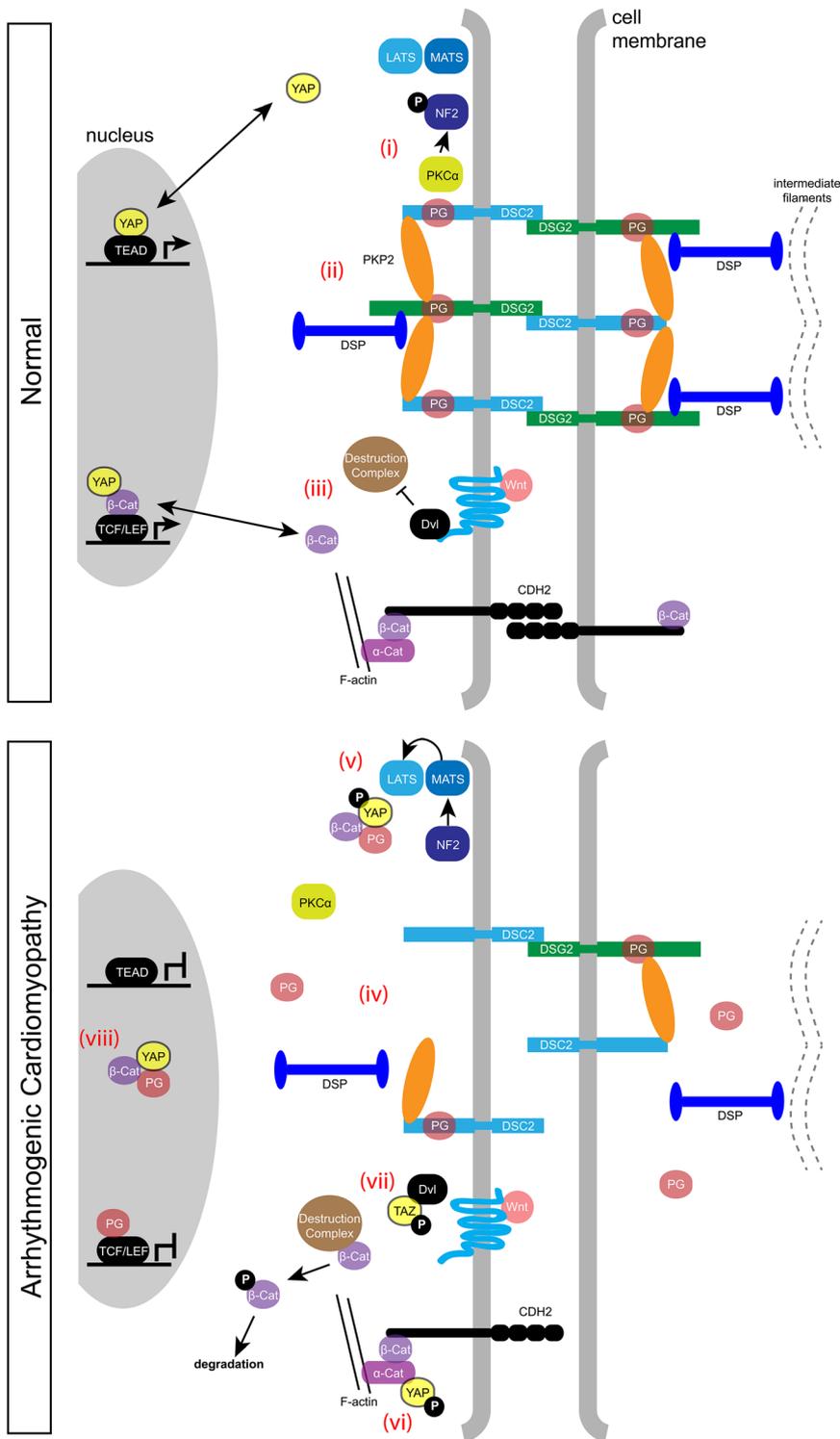
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From the Department of Cardiology, Children's Hospital Boston, Boston, MA (Y.H., W.T.P.); and Harvard Stem Cell Institute, Harvard University, Cambridge, MA (W.T.P.).

Correspondence to William T. Pu, MD, Department of Cardiology, Children's Hospital Boston, Enders 1254, 300 Longwood Ave, Boston, MA 02115. E-mail wpu@enders.tch.harvard.edu  
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**Figure. Potential interaction of Hippo/YAP and Wnt/β-catenin signaling pathways in arrhythmic cardiomyopathy (AC).** **Top**, Normal myocardial intercalated disk (ID), containing desmosomes (Desmoglein-2 [DSG2]/DSC2/Plakophilin-2 [PKP2]/plakoglobin [PG]/DSP) and adherens junctions (CDH2/β-catenin/α-catenin). The intact ID retains PKCα near the membrane, leading to phosphorylation and inactivation of NF2 (i). YAP phosphorylation state is low, and PG is bound to desmosomes and localized at the ID (ii). Wnt/β-catenin signaling can proceed normally (iii). **Bottom**, Disruption of the ID in AC by a desmosomal mutation (eg, in PKP2; iv). ID disruption causes both loss of mechanical integrity and aberrant signaling. Loss of PKCα submembrane localization activates NF2 and the Hippo kinase cascade (MST and LATS; v). As a result, YAP is phosphorylated and localizes to IDs, perhaps by binding to α-catenin (vi). Phosphorylated YAP may also bind to Dvl and thereby activate APC to enhance β-catenin degradation (vii). Nuclear PG, perhaps in the form of a complex with β-catenin and YAP, interferes with nuclear β-catenin and possible YAP transcriptional activity (viii). This complex may also have nontranscriptional roles in the cytoplasm or near IDs. Dvl indicates disheveled; LEF, lymphoid enhancer-binding factor-1; TCF, T-cell factor; and TEAD, TEA domain family member 1.

activate the transcriptional activity of YAP-TBX5 transcriptional complexes,<sup>17</sup> introducing a further mode of crosstalk between the Wnt and Hippo pathways.

Because of the reported crosstalk between Wnt/β-catenin and Hippo/YAP signaling pathways, Chen et al<sup>8</sup> investigated the Wnt/β-catenin signaling pathway in AC. In human AC myocardium, total β-catenin levels were unchanged, but it was no longer well localized to IDs.<sup>8</sup> Phosphorylated β-catenin (inactivated β-catenin targeted for degradation) was

higher in AC myocardium. Similar changes in β-catenin levels were also observed in murine AC myocardium, although it remained localized to IDs in these models. Altered YAP or β-catenin localization to the nucleus was not detected by immunofluorescent staining of human or murine AC myocardium, although this method can be insensitive to reduction of a weak signal. Indeed, transcriptomic analysis was consistent with reduced Wnt/β-catenin transcriptional activity. These data suggest a somewhat paradoxical situation in which

destabilization of IDs in AC correlated with mobilization of  $\beta$ -catenin away from adhesion junctions and yet a diminution of transcriptionally active  $\beta$ -catenin. Potentially these observations might be rationalized by a combination of increased  $\beta$ -catenin phosphorylation/inactivation and inhibition of  $\beta$ -catenin activity by mobilized PG. Overall, the data point to reduced Wnt/ $\beta$ -catenin and Hippo/YAP signaling (greater Hippo kinase activity leading to inhibition of YAP) in AC.

Chen et al<sup>8</sup> identified a new potential source of crosstalk between Wnt/ $\beta$ -catenin and Hippo/YAP signaling pathways in AC, namely the formation of protein complex(es) between YAP, PG, and  $\beta$ -catenin. Immunoprecipitation of YAP coprecipitated both  $\beta$ -catenin and PG. However, whether these proteins exist in 1 complex or in distinct complexes, and the subcellular localization of these complexes, remains to be determined. The functional significance of the newly described YAP–PG interaction with respect to Hippo/YAP or Wnt/ $\beta$ -catenin signaling likewise requires further study.

To assess the functional significance of Hippo signaling on the development of AC phenotypes, Chen et al<sup>8</sup> evaluated HL-1 cells with shRNA-mediated knockdown of PKP2 and LATS1/2. PKP2 knockdown promoted formation of intracellular fat droplets and increased the fraction of cells expressing the adipogenic transcription factor PPAR $\gamma$ . These effects were antagonized by knockdown of the Hippo kinases. Thus, these data are consistent with Hippo kinase activation promoting adipogenesis in AC.

Like all scientific advances, the study raises new questions. Among them: (1) What AC genotypes are characterized by Hippo kinase activation? The study of Chen et al<sup>8</sup> unfortunately did not disclose the AC genotypes of the myocardial samples studied. Are these findings observed in all patients with desmosomal gene mutation? How about those patients with AC and without identified desmosome gene mutation? More information is also needed to determine the heart disease stage associated with Hippo kinase activation. The human samples used in this study were from patients with advanced AC, whereas the relationship of disease severity to Hippo kinase activation was not systematically interrogated in the murine models. (2) How does Hippo kinase activation influence AC phenotypes (arrhythmogenesis; lipogenesis; myocardial fibrosis impaired cardiac contraction) in vivo? Although the study of Chen et al demonstrated activation of Hippo kinases and YAP phosphorylation in human AC and murine AC models, its pathogenic consequences in AC await further investigation. (3) What is the cardiac cell type that gives rise to adipocytes? Although transdifferentiation of cardiomyocytes<sup>18</sup> and altered differentiation of cardiac progenitor cells<sup>6</sup> have been proposed to yield adipocytes observed in AC, at present the origin of these cells remains unclear. Determining the contribution of Hippo kinase activation to adipogenesis in this disease awaits resolving this mystery. (4) What is the functional crosstalk between Wnt/ $\beta$ -catenin and Hippo/YAP pathways in AC, and how does this crosstalk contribute to the molecular pathogenesis of this disease? Does this crosstalk mediate changes in  $\beta$ -catenin localization and phosphorylation in AC, as described by Chen et al? (5) What is the nature and role of complex(es) containing PG, YAP, and  $\beta$ -catenin? Does PG interact simultaneously

with both YAP and  $\beta$ -catenin, or are the complexes mutually exclusive (and competitive)? How do these complexes affect Wnt/ $\beta$ -catenin signaling? How do they affect YAP signaling through its partner transcription factors TEAD or TBX5? Do these complexes have nontranscriptional roles, for example, do they modulate signaling at adhesion junctions?

In summary, the work of Chen et al<sup>8</sup> highlights the key signaling role of IDs in cardiomyocytes and mechanistically links desmosome disruption to Hippo signaling through a novel upstream Hippo regulatory mechanism. It identifies a novel new PG–YAP interaction that may connect desmosomes to both Hippo/YAP and Wnt/ $\beta$ -catenin signaling pathways. In doing so, the study opens exciting new avenues to understand the pathogenesis of this enigmatic disease.

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### Disclosures

None.

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