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Coins of the Realm in Atrioventricular Junction Development:

An editorial comment on: Canonical Wnt Signaling Regulates Atrioventricular Junction Programming and Electrophysiological Properties (CR-304731)

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The development of nodal and working myocardium involves a complex signaling cascade ultimately resulting in the production of fast conducting myocardium from a developmental predecessor pacemaking phenotype. Understanding this signaling network has profound implications for understanding normal cardiac development, complex congenital heart diseases as well as development and utilization of progenitor cells as disease models and regenerative therapeutics such as biological pacemaking.

The mature mammalian atrioventricular node (AVN) and junction is an anatomically complex, cellularly heterogeneous structure that normally constitutes the only communication between the atrium and ventricular myocardium.^{1, 2} The intricate structure is commensurate with an equally complicated physiological phenotype, in part predicted by that structural complexity and involved in a number of important cardiac arrhythmias.³ The embryonic atrioventricular canal (AVC) appears to form not only components of the AV conduction system but also working myocardium adjacent to the AV ring,⁴ some of these tissues such as that forming the coronary sinus ostium may themselves have pacemaking properties and be the source clinical arrhythmias.

The signaling pathways that govern AV junctional structure and function are spatially and temporally regulated and a general consensus has emerged that includes repression by specific T-box transcription factors (*Tbx2/3*) that help to maintain a pacemaking phenotype⁴ and interact with other upstream regulators such as *Smads/Bmp2* to restrict the expression of *Tbx2/3* to tissues destined to be AVC and AVN. Contemporaneously there is migration of epicardial derived cells that undergo epithelial to mesenchymal transition to form the electrically insulating annulus fibrosis, a process that may not be completed by birth.^{5, 6} In addition to inducing proliferative responses in progenitor cells, Notch signaling appears to be involved in the transition of portions of the embryonic AVC myocardium into mature AVN myocardium.⁷

A body of research is accumulating that has helped to understand the molecular signaling pathways involved in AVC myocardial specification and AVN formation. In previous work, Rentschler and co-workers described the effect of Notch activation on the development of

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ectopic AVC myocardium and the apparent generation of ventricular preexcitation in the mouse.⁷ Notch is a conserved transmembrane protein and its canonical signaling is activated by binding of extracellular ligands to Notch, which leads to intracellular cleavage and translocation of the Notch intracellular domain (NICD) into the nucleus where it associates with the transcription factor RBP-J and co-activators for gene activation⁸. Inhibition of Notch signaling using a dominant negative form of mastermind-like protein—a co-activator necessary for canonical Notch signaling—resulted in the failure to form a mature AVN. Myocardial activation of Notch signaling by overexpressing NICD produced a spatially restricted increase in myocardial tissue along the right AV junction as well as epicardial muscle sleeve that spans a relatively intact annulus fibrosis and that expresses atrial and ventricular markers on the respective chamber sides of this sleeve. The tissue electrophysiology suggests that this is working myocardium with rapid conduction. The mice exhibit short PR and atrioventricular (AV) conduction intervals as well as 1:1 ventricular capture with rapid pacing from the atrium consistent with ventricular preexcitation. Thus the steps to an accessory AV pathway (AP) are persistence or development of an AV communication with progression to a working myocardium electrical phenotype and incomplete insulation by the annulus fibrosis. The failure to develop a muscular phenotype in an AV connection may be associated with the development of a specific types of APs, known as Mahaim fibers (atriofascicular or atrioventricular dependent upon the distal site of insertion into the myocardium) that exhibit conduction tissue properties such as decremental conduction⁹ and uniformly span the tricuspid valve annulus where AVC tissue is more abundant.¹⁰ While Notch frequently intersects with canonical Wnt (Wnt/ β -catenin) signaling during development¹¹ and proper AVC patterning requires Wnt signals¹², it was unclear if Wnt/ β -catenin signaling influences programming and function of AV junctional cells.

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In the manuscript by Gillers *et al.*¹³ in this issue of the journal, they further evaluate the role of Wnt/ β -catenin signaling in the development of the AV junction. By conditionally expressing a β -catenin allele (*Ctnnb1*^{dm}) that lacks Wnt-mediated transcriptional activity but preserves its cell adhesion properties in cardiomyocytes (*Ctnnb1*^{dm/fl}, a.k.a. Wnt LOF) they observed increased perinatal lethality with both right ventricular (RV) and tricuspid valve (TV) defects. Although they expressed *Ctnnb1*^{dm} broadly in myocardium (likely due to the unavailability of an AVC myocardium-specific Cre driver), the valvular defects are not likely to be a secondary phenotype from defective ventricular formation as Wnt LOF or complete elimination of β -catenin conducted with a ventricular myocyte-specific Cre driver, which minimally affects AV junction myocytes, results in defective right heart development with intact AV valves. Defective Wnt signaling appears to prevent the maintenance AVC programming; at earlier stages of AVC development, cardiac morphology and gene expression of *Tbx3* and *Bmp2* are preserved. They next examined whether canonical Wnt signaling is sufficient to generate ectopic AV junctions using a transcriptionally active form of β -catenin (*Ctnnb1*^{fl(ex3)}, Wnt GOF mice) that lacks glycogen synthase kinase 3 β phosphorylation sites required for proteosomal degradation. Remarkably the mice exhibit regions scattered throughout the ventricles that structurally resemble AV junctions including coronary vasculature, epicardially-derived adipocytes, and fibroblasts. Moreover, Wnt GOF down-regulates *Scn5a* and *Cx43* in these regions of the ventricles while AVC enriched

genes such as *Tbx3* and periostin (seen in developing annulus fibrosus) are up-regulated. The functional consequences of ectopic Wnt activation included slower conduction velocities reflected as prolongation of the PR and QRS duration. Conduction slowing was present in both ventricles but was more prominent in the RV.

The relationship of postnatal Notch and Wnt signaling in the functional development of the myocardium was studied using an inducible Notch expression system in mice. Increased Notch levels in postnatal myocytes down-regulate Wnt/ β -catenin signaling and reprogram *Tbx3*⁺/*Nav1.5*⁻ AV junction myocardium to *Tbx3*⁻/*Nav1.5*⁺ chamber-like myocardium. It is worth noting that Notch can down regulate Wnt target genes without affecting β -catenin transcripts, suggesting a post-translational regulation. The AP development and ventricular preexcitation observed in Notch (NICD) activated mice can be rescued in the progeny of crosses with Wnt GOF mice. The authors conclude that inhibition of canonical Wnt signaling is necessary but not sufficient for the development of ventricular preexcitation and that Notch-mediated effects that give rise to preexcitation are in part the result of canonical Wnt down regulation. It is likely that defects in both signaling pathways are required for the generation of the APs that constitute the substrate for Wolff-Parkinson-White syndrome.

A number of interesting questions arise from this work. Prominently is the mechanism of the right heart predilection particularly for the effects of the Wnt GOF. Is this a consequence of the differences in embryological origin of the tissue that produces inherent functional variation RV and LV myocardium? Are the Notch/Wnt signaling targets different in RV compared to LV myocardium?^{14, 15} What is the relationship of the RV prominence of alterations in Wnt signaling to diseases that preferentially affect the right heart such as TV atresia, hypoplastic RV, Brugada syndrome and arrhythmogenic right ventricular dysplasia/cardiomyopathy? It will be also important to understand the mechanisms by which Notch regulates Wnt/ β -catenin signaling in this context as growing evidence suggests that Notch can indeed post-transcriptionally regulate Wnt/ β -catenin signaling by targeting the active form of β -catenin.¹⁶

The role and mechanism of spatial restriction of Notch and Wnt signaling in the generation of APs, which in most circumstances tend to be restricted and most often are, with the exception of Ebstein anomaly, left sided. The role of Notch and Wnt in modulation of the electrophysiological function of the myocardium in general and APs specifically is intriguing. Is the refractory period of an AP determined by signaling mechanisms operative during development? Despite the presence of techniques to destroy such tracts effectively and safely by catheter ablation, are there ways to modulate Wnt/Notch signaling to produce changes in AP refractoriness to render them functionally inconsequential? In many ways Wnt and Notch signaling pathway are important coins in the realm of in the understanding and managing AV conduction abnormalities.

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