Article Addendum

Ankyrin-based targeting pathway regulates human sinoatrial node automaticity

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Cellular defects in ankyrin-based ion channels and transporter targeting pathways have previously been linked with abnormal vertebrate physiology and human disease. In a recent study, our group linked dysfunction in cardiac ankyrin-B function with human sinus node disease. Ankyrin-B deficient mice displayed bradycardia and heart rate variability similar to individuals harboring an ANK2 variant. Isolated sinoatrial node (SAN) cells from ankyrin-B-deficient animals displayed abnormal membrane expression of Na+/Ca2+ exchanger (NCX1), Na+/K+ ATPase (NKA), IP3 receptor (IP3R) and, surprisingly, CaV1.3. Loss of ankyrin-B promoted slow and irregular Ca2+ release, as well as afterdepolarizations in isolated SAN cardiomyocytes. Our findings suggest that ankyrin-B serves as a critical focal point for channels and transporters important for sarcoplasmic reticulum (SR) calcium homeostasis as well as membrane depolarization in SAN cells. The severity and penetrance of human ANK2 sinus node dysfunction likely reflects the essential role of ankyrin-B for orchestrating membrane function of multiple SAN ion channel and transporters within a single functional pathway. Therefore, ankyrin-based pathways may serve as ideal therapeutic targets in SAN cardiomyocytes where a “multi-hit” approach is necessary to impact a complex process such as SAN cell automaticity. In summary, our new findings define a novel genetic basis for human SND and expand our understanding of the critical role that ankyrin-based targeting pathways play in excitable cell physiology.

Each normal human heartbeat begins as a spontaneous action potential generated from within the sinoatrial node (SAN), a compact collection of specialized excitable cardiomyocytes in the right atrium.1 SAN cells are uniquely equipped with structural and electrophysiological properties that allow for spontaneous and repetitive depolarization of the SAN cell membrane. Despite over 40 years of investigation, our understanding of pacemaking mechanisms in the human sinus node remains incomplete.1 However, it is clear that cardiac pacemaking is a complex process initiated at the level of the single cell by the coordinate activity of depolarizing and repolarizing currents (e.g., If (HCN4), IBK (Na+/Ca2+ exchanger) and Ica (CaV1.2/1.3 and CaV3.1/3.2)), as well as intracellular ion fluxes (RyR2).2 Ankyrins are adapter proteins that link critical membrane proteins (e.g., ion channels, transporters, cell adhesion molecules) to the actin/spectrin-based cytoskeleton.3 Work from several groups has identified a close association between ankyrin dysfunction and human disease. For example, mutations in ANK1 (encodes ankyrin-R) cause sphero-cytosis and hemolytic anemia,4,5 while loss-of-function variants in ANK2 (encodes ankyrin-B)6 are associated with catecholaminergic polymorphic ventricular tachycardia, conduction defects, syncope and sudden death.6,8 Finally, dysfunction in the ankyrin-G(ANK3)-based pathway for voltage-gated sodium channel (NaV1.5) targeting in ventricular cardiomyocytes is associated with Brugada syndrome arrhythmia.9,10

In a recent study,11 our group linked dysfunction in cardiac ankyrin-B function with human sinus node disease. Specifically, we identified ANK2 allele variants associated with sinus node dysfunction (SND) in two families in France: a family of 74 individuals, 26 of whom had SND, and a family of 44 individuals, 13 of whom had the disease.11 Affected individuals in the first kindred carried an ANK2 gene missense variant and experienced variable heart rate and severe bradycardia. Ankyrin-B deficient mice also displayed bradycardia and heart rate variability similar to individuals harboring the ANK2 variant. Moreover, isolated SAN cells from ankyrin-B-deficient animals displayed abnormal membrane expression of the Na+/Ca2+ exchanger (NCX1), Na+/K+ ATPase (NKA), IP3 receptor (IP3R) and, surprisingly, CaV1.3.11 Consistent with these changes in cell surface expression, we measured reduced NCX and Ca2+ current in ankyrin-B deficient SAN cells. Finally, loss of ankyrin-B promoted slow and irregular Ca2+ release, as well as afterdepolarizations in isolated SAN cardiomyocytes.

Aberrant SAN electrical activity and altered Ca2+ transients observed in ankyrin-B deficient SAN cells are likely due to aberrant
membrane expression of Cav1.3 and NCX1 and corresponding reduction of both I_{Na} and I_{Ca,L}.

Moreover, increased cytosolic [Na\(^{+}\)] caused by reduced NKA in ankyrin-B-deficient SAN cells likely contributes to cellular defects in SAN calcium transients and excitability by promoting further inhibition of NCX1 activity. Definitive roles for SAN IP_{3}R3s have not been well established. However, periodic IP_{3}R activity underlies initiation of pacemaking and differentiation of embryonic cardiomyocytes. Therefore, the combined molecular dysfunction of the ankyrin-B-based targeting pathway creates a complex electrical phenotype due to improper membrane targeting of multiple proteins.

The absolute requirement of SAN function for vertebrate survival has likely resulted in the evolution of redundant molecular pathways to regulate SAN excitability (i.e., HCN4, NCX1, Cav1.2/Cav1.3, RyR2/RyR3). The severity and penetrance of human ANK2 SND likely reflects the essential role of ankyrin-B for orchestrating the membrane function of multiple SAN ion channel and transporters within a single functional pathway. Thus, the “single hit” mutation in ANK2 orchestrates a “multi-hit” loss of NCX1, Na\(^{+}/K\(^{+}\) ATPase, Cav1.3 and IP_{3}R.

Previous studies have shown that ankyrin-B binds to and targets NCX1, NKA and IP_{3}R in ventricular cardiomyocytes. Apparently, ankyrin-based pathways have evolved in distinct cell types (e.g., ventricular vs. SAN cells) to mediate very different primary functions (e.g., contraction vs. automaticity). While there is overlap in ion channels and transporters targeted by ankyrin-B in ventricular and SAN cardiomyocytes (e.g., NCX1, NKA and IP_{3}R), Cav1.3 exists as a unique member of the ankyrin-B targeting pathway in SAN cells (Fig. 1). As a consequence, ankyrin-B serves as a focal point for channels and transporters important for sarcoplasmic reticulum (SR) calcium homeostasis as well as membrane depolarization. Recently, a great deal of attention in the field of molecular cardiology has focused on whether SAN automaticity is driven primarily by membrane depolarization (“membrane voltage clock”) or by intracellular calcium cycling (“calcium clock”). Our new findings identify ankyrin as a critical link between ion homeostasis, SR calcium cycling and membrane depolarization in SAN cells. It is intriguing to consider the possibility that ankyrin-B organizes local domains at the cell membrane and facilitates coordination of the “membrane voltage clock” and SR “calcium clock.” The fact that dysfunction in ANK2 results in SND suggests that uncoupling of these cellular processes may be necessary and sufficient to precipitate arrhythmia. Finally, based on the critical role of Cav1.3 in SAN and aberrant targeting of Cav1.3 in mice SAN cells lacking ankyrin-B, it will be critical in future experiments to characterize Cav1.3 as a novel ankyrin-binding protein (Fig. 1).

Our new findings on the role of ankyrin-B in regulating SAN function have implications beyond the molecular mechanism underlying a novel form of human SND. While the human gene variants identified in our study are rare, it is important to emphasize that SND is nearly completely penetrant in individuals with ANK2 linkage and is observed for all ages, including in utero. Moreover, recent findings have linked a major locus for resting heart rate in the general population to a site on human chromosome 4q that overlaps ANK2. These exciting findings suggest that ANK2 dysfunction may play a role in the genesis of SND and sudden death in the general human population.

In summary, our recent study defines a novel genetic basis for human SND and expands our understanding of the critical role that ankyrin-based targeting pathways play in excitable cell physiology. Human SND caused by ankyrin-B dysfunction is the first example of SND involving a non-ion channel protein and dysfunction in intracellular calcium regulation. Moreover, this new form of SAN disease, based on abnormal ion channel and transporter targeting, elucidates the importance of local membrane organization for SAN pacemaking. As heart rate is an independent risk factor for mortality,23 these new findings identify an unexpected cardiovascular disease susceptibility gene in ANK2. Ankyrin-based pathways may serve as ideal therapeutic targets in excitable cells such as SAN cardiomyocytes where a “multi-hit” approach is necessary to impact a complex and redundant process such as SAN cell automaticity.

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