Cytoskeleton Regulation of Ion Channels

Running title: Fu et al.; Cytoskeleton Regulation of Ion Channels

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Cardiac arrhythmia is one of the leading causes of sudden death in developed countries, and results from abnormal cardiac electrical activity. Regular beat-to-beat cardiac excitability requires the coordinated activity of membrane bound ionophoric proteins which are channels and transporters. Aberrant currents can result from either changes in the gating properties of ionophores or altered trafficking to their appropriate membrane subdomain. Mutations in the genes of ionophoric proteins result in inherited human arrhythmogenic syndromes. While mutations of channel proteins can affect biophysical gating properties, most inherited arrhythmia disorders likely result from altered protein trafficking rather than gating. In addition, mutations in the proteins that make up the trafficking apparatus, namely the cytoskeletal and associated proteins, can also result in arrhythmia disorders.

Ankyrins are an important family of cytoskeleton related adapter proteins known for localizing proteins to specialized subdomains in a variety of cells types. Ankyrins interact with ionophoric proteins, whereas their central domain binds to members of the β-spectrin family of actin-associated cytoskeleton, thereby linking the channel to the actin cytoskeleton. Ankyrins in the vertebrate heart are encoded by three genes: ankyrin-R (ANK1), ankyrin-B (ANK2) and ankyrin-G (ANK3), with the ankyrin-B gene product being critical for localizing the Na/Ca exchanger and Na/K ATPase to the plasma membrane and inositol 1,4,5 trisphosphate (IP3) receptor to the sarcoplasmic reticulum. The study in the current issue by Smith et al reports a novel human mutation in ankyrin-B (ANK2) gene that results in recurrent ventricular fibrillation. It is a single amino acid substitution at a highly conserved residue p.R990Q, which locates at a spectrin binding domain on ankyrin-B that is far upstream of the ankyrin-B C-terminal domains, where all previous mutations were identified. Although in silico modeling shows that the mutation is not on the interaction interface between ankyrin-B and βII-spectrin, binding of βII-
spectrin to the mutated p.R990Q ankyrin-B is greatly impaired when tested in vitro. Therefore, the authors proposed a model in which βII-spectrin recruits ankyrin-B and targets ionophore proteins to the membrane, regulating cardiac excitability. The novelty in this finding relates to the independence of the mutation to ionophore proteins. Not only is the mutation in a cytoskeleton adaptor protein (ankyrin-B), but it affects a cytoskeletal attachment domain rather than an ion channel binding domain, much in the way that mutations in the structural protein desmoplakin affects binding of the microtubule plus-end protein EB1, affecting Connexin43 delivery to intercalated discs.8

The proband in the study by Smith et al7 in this issue of Circulation was reported to have a prolonged QTc. Causality of the p.R990Q mutation in ankyrin-B was established primarily by examining the phenotype of a mouse model with cardiac specific deletion of βII-spectrin. The assumption in the spectrin knockout model is that removing spectrin is the functional equivalent of a mutation decreasing spectrin’s ability to bind ankyrin-B. The knockout mouse recapitulated long QT, and also was notable for nodal dysfunction including bradycardia, AV block, and stress-induced ventricular ectopy. In addition, the authors found that the ryanodine receptor (RyR2) protein was dramatically reduced due to loss of βII-spectrin, and imaging indicated smaller RyR2 clusters as well. These findings help explain the aberrant calcium handling observed in isolated cardiomyocytes and in vivo arrhythmogenic phenotype. The study also demonstrated changes in previously known molecular partners of ankyrin-B such as Na/Ca exchanger and Na/K ATPase, thus highlighting the significance of βII-spectrin/ankyrin-B cytoskeletal complex in vivo. Overall, the data strongly support an important role of βII-spectrin in maintaining ionophore integrity required for electrical health of the heart.

In the field of cardiac myopathy and dystrophy, the role of cytoskeleton proteins in
maintaining membrane protein integrity of cardiomyocytes has been well accepted. However, the importance of the cytoskeleton in organizing local membrane microdomains required for electrical integrity remains underappreciated. Smith et al\(^7\) provide strong imaging and biochemical evidence supporting that βII-spectrin binds to ankyrin-B, regulating expression levels and organizing ionophoric membrane proteins, RyR2, and cytoskeleton proteins such as other ankyrins as well as α-spectrin and α-tubulin. In sum, βII-spectrin is an important protein in ventricular cardiomyocytes helping regulate excitability and excitation-contraction coupling.

Despite the clear importance of the findings and identification of the role of βII-spectrin in multiple cardiomyocyte functions, there are several questions to resolve before βII-spectrin is enshrined as an important candidate molecule for inherited human arrhythmia. The proband in Smith et al\(^7\) had a mutation in ankyrin-B, not βII-spectrin. There was limited clinical phenotyping of the proband, and no discussion of possible affected relatives. The animal model used to recapitulate the proband’s phenotype involved a complete deletion of βII-spectrin in the heart, which is a larger genetic intervention than a single point mutation in ANK2. A more applicable animal model would have been a mouse R990Q knock-in to the ANK2 gene, not total cardiac knockout of the ankyrin-B binding partner, βII-spectrin. In fact, knockout of βII-spectrin resulted in several severe effects, including ironically a large reduction in ankyrin-B expression and increase in ankyrin-G expression, which together will affect multiple ion channels, again suggesting spectrin’s role as an important accessory to ankyrins. Although the authors tested cellular functions of the R990Q mutation in vitro, the studies were limited to the rescue of Na/Ca exchanger localization in differentiated neonatal cardiomyocytes. Experiments evaluating the ANK2 R990Q mutation’s ability to reproduce the proband’s long QT phenotype could not be performed. The electrical phenotype of β-spectrin cKO mice did have long QT and also included
bradycardia and heart block which point to significant impairment in the conduction system as well. It was not mentioned whether the proband had conduction system defects. Similarly, the ECG of βII-spectrin cKO animal subjected to aortic banding also showed arrhythmias primarily due to AV block rather than ventricular arrhythmias which is inconsistent with the reported human proband.

With regards to trafficking, the authors report that βII-spectrin plays central roles in organizing membrane proteins. Caution should be used in presuming trafficking involves forward trafficking only, and not considering potential intramembrane movements or internalization (retrograde trafficking). A thorough characterization of dynamics of protein trafficking in which forward, intramembrane, and retrograde trafficking are isolated and examined in dynamic detail will help elucidate the mechanisms by which βII-spectrin affects ion channel localization. For instance, according to the Targeted Delivery paradigm, both Connexin43 and the Cav1.2 are forward trafficked using microtubule highways, and membrane scaffolds, and Connexin43 is internalized by a cascade of phosphorylation events leading to ubiquitination. Do ankyrin-B and βII-spectrin regulate forward trafficking or internalization?

Despite the severity of arrhythmias associated with p.R990Q mutation in ankyrin-B, this is a rare mutation with allele frequency of ~0.007%. Key trafficking proteins that change in acquired heart failure may be causally associated with common arrhythmias and commonly occurring decreased excitation-contraction coupling. For instance, EB1, which aids Connexin43 forward trafficking, is displaced from microtubules in acquired heart failure, decreasing Connexin43 delivery to the intercalated disc. Also the actin and microtubule anchoring protein BIN1, which also folds T-tubule membrane and facilitates forward delivery of Cav1.2 channels, is decreased in failing human and animal heart. Fortunately, with BIN1, its
expression recovers with recovery of heart function\textsuperscript{17}. The human mutation in ANK2 gene and study by Smith et al\textsuperscript{7} reveal that βII-spectrin is important and highlights critical roles of βII-spectrin in cardiomyocyte biology. The clinical impact of βII-spectrin will be considerably increased if its expression levels or function are negatively affected by non-inherited, acquired heart failure. The change in α-tubulin concentration with βII-spectrin knockdown\textsuperscript{7} suggests that if βII-spectrin is diminished in acquired heart failure, it may also affect ionophore trafficking.

Smith et al\textsuperscript{7} are to be congratulated on advancing ankyrin-B studies to not only finding that mutations of its cytoskeleton attachment domains can be related to human arrhythmia, but that the ankyrin-B/βII-spectrin interaction underlies critical ionophore protein localization and expression levels of key excitation-contraction coupling proteins. As a result, we speculate, and frankly encourage, greater interest in cytoskeleton regulation of electrophysiology. As more is learned about the complex interaction between cytoskeletal and ionophore proteins, the lessons learned will be applied not just to patients with inherited disease, but to the common and growing number of patients with acquired heart failure.

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**References:**


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