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Nexilin: a new player for shaping T-tubules in cardiomyocytes

Jinxi Wang, Ph.D.[1], **Duane D. Hall, Ph.D.**[1], **Long-Sheng Song, M.D., M.S.**[1,2,3]

[1]Division of Cardiovascular Medicine, Department of Internal Medicine, Abboud Cardiovascular Research Center, Carver College of Medicine, University of Iowa, Iowa City, IA 52242;

[2]Fraternal Order of Eagles Diabetes Research Center, Carver College of Medicine, University of Iowa, Iowa City, IA 52242;

[3]Department of Veterans Affairs, Iowa City Medical Center, IA 52242

Heart disease and failure typically arise from the heart's inability to meet systemic oxygen demands. During disease progression, working cardiomyocytes are less able to generate the rigorous force required for the heart to pump blood to the whole body. Action potentials during each heartbeat are normally coupled with uniform Ca^{2+}-dependent myofilament contraction, known as Excitation-Contraction (E-C) coupling, and is governed through a process called Ca^{2+}-induced Ca^{2+} release.[1] To achieve an efficient and synchronous Ca^{2+} release throughout the cytosol, cardiomyocytes have developed an exquisite network of specialized membrane structure known as transverse tubules, or T-tubules.[2, 3] During early postnatal cardiac development, T-tubules originate from the sarcolemma along sarcomeric Z-lines that begin to invaginate and project interiorly. As T-tubules mature, they concentrate L-type Ca^{2+} channels required for Ca^{2+} influx and form stable contacts with nearby sarcoplasmic reticulum (SR) membranes abundant with type 2 ryanodine receptor (RyR2) Ca^{2+} release channels. These T-tubule/SR contact sites are referred to as cardiac dyads or junctional membrane complexes (JMCs).[4, 5] Thus, cardiomyocyte maturation results in regularly arrayed Ca^{2+} crosstalk microdomains, facilitated by an intricate T-tubular network, that provides the physical basis for simultaneous and concerted intracellular Ca^{2+} spikes required for each heartbeat. The importance of maintaining dyad structure is evident in the hypertrophied and failing heart as T-tubule remodeling and ensuing uncoupling from junctional SR constitute the ultrastructural basis of E-C uncoupling and Ca^{2+} handling dysfunction in heart disease.[6, 7] Even though much is known regarding the structure and function of the T-tubule system in both health and disease, what remains incompletely understood is how the articulately invaginated T-tubule is initiated and formed, and how the JMC itself is regulated in cardiomyocytes.

Decades of research have endeavored to identify JMC proteins essential for establishing and stabilizing the cardiac dyad. Junctophilin-2 was the first and only junctophilin family protein found to be responsible for forming JMCs in cardiomyocytes.[5] It is now recognized that

Corresponding Author: Long-Sheng Song, M.D., M.S., FAHA, Division of Cardiovascular Medicine, Department of Internal Medicine, Abboud Cardiovascular Research Center, Carver College of Medicine, University of Iowa, 285 Newton Road, Iowa City, IA 52242, Phone: (319) 384-2890, Fax: (319) 353-5552, long-sheng-song@uiowa.edu.

Disclosure

None.

junctophilin-2 is also required for maintaining T-tubule integrity in a regularly organized network, especially under stress conditions.[8, 9] BIN1 is thought to be the key protein involved in organizing JMCs by facilitating localization of L-type Ca^{2+} channels to T-tubules as well as in forming micro-infolds along T-tubules.[2] Nexilin (NEXN) has been identified as an actin binding and Z-disk protein. Multiple mutations in the *NEXN* gene are related with both hypertrophic and dilated cardiomyopathy,[10, 11] but the precise role of NEXN in heart function and disease is still unknown.

In a recent article by Chen and colleagues,[12] the authors generated global and cardiomyocyte specific *Nexn* knockout (KO) mice and found both models develop rapidly progressive dilated cardiomyopathy and postnatal lethality by two weeks of age. Developing cardiomyocytes of *Nexn* KO animals did not form T-tubules likely from a failure to initiate sarcolemmal invagination. In addition, their results indicate NEXN regulates JMC function at multiple levels. NEXN was shown to interact with RyR2 and junctophilin-2 junctional SR proteins and KO hearts had altered expression of key dyadic proteins and significant Ca^{2+} handling defects. Although this study revealed that NEXN is a new component of JMCs and is required for T-tubule initiation and formation during development, the early postnatal lethality phenotype in these KO models are prohibitive for understanding whether NEXN plays a central role in T-tubule function in the adult heart or during pathological cardiac remodeling following stress.

In this issue of *Circulation: Heart Failure*, Chen and colleagues build on their initial findings by generating a *Nexn* inducible cardiomyocyte-specific knockout (icKO) mouse model.[13] This new mouse model allowed them to study the role of NEXN in adult hearts and mature cardiomyocytes consisting of a fully developed and functional T-tubule network. Through morphological and functional analyses, the authors show that *Nexn* icKO in adult mice for two weeks also promotes dilated cardiomyopathy with reduced cardiac function and increased mass, fibrosis and hypertrophic gene expression. The authors revealed that NEXN is not only essential for optimal calcium handling in mature myocytes, similar to that found in global and cardiac KO mice, but also for normal contractile function. Adult *Nexn* icKO cardiomyocytes have reduced shortening and prolonged relaxation during contraction that correlates with decreased Ca^{2+} transient amplitude and impaired cytosolic Ca^{2+} clearance. These phenomena are consistent with reduced expression in RyR2 and Serca2 junctional SR and Ca^{2+} handling proteins and mis-localization of L-type Ca^{2+} channels away from the Z-lines after *Nexn* icKO. It is worth noting that both studies identified a surprising and significant upregulation of skeletal muscle expressed Casq1 protein in icKO hearts, instead of the cardiac isoform Casq2. This suggests SR Ca^{2+} buffering activity may be modified in icKO hearts and merits future investigation. The authors also examined the role of NEXN in T-tubule organization and found *Nexn* icKO significantly reduced the transversal, but not axial, component of T-tubule system. These results revealed NEXN as a pivotal component of adult cardiomyocyte JMCs that is required for the maintenance of higher order T-tubule architecture and JMC integrity. Thus, this study provides a novel role for NEXN in the adult cardiomyocyte and gives further understanding of the pathological mechanisms responsible for cardiomyopathy in patients with NEXN mutations.

An important question remains to be addressed: what is the exact mechanism of NEXN in maintaining T-tubule architecture and function? As NEXN is a component of the JMC by binding to junctophilin-2 and RyR2,[12] more biochemical studies are warranted to better understand the precise molecular interactions between NEXN and JMC proteins necessary for sarcolemma/SR tethering and normal operation of Ca^{2+}-induced Ca^{2+} release. Additionally, further studies are needed to precisely determine how specific alterations in NEXN sequence modify protein function and cause cardiac disease. As NEXN mutations can promote either hypertrophic or dilated cardiomyopathy, it will be of great interest to define the molecular structure and function of NEXN, and possible NEXN gain- and loss-of-function mechanisms.

Altogether, the works of Chen's group identify NEXN as a new component of JMCs in cardiomyocytes that is essential for T-tubule initiation and formation during development. In adult hearts and cardiomyocytes, NEXN is also necessary for maintaining the T-tubule system. It will be important to determine whether NEXN also plays a central role in T-tubule/JMC remodeling during heart failure stress responses, even though there is no significant change in patients with heart failure.[12] It is worthwhile seeing whether posttranscriptional modifications that occur during heart failure can modulate NEXN function, possibly by regulating its known actin-binding activity. We previously showed that actin-dependent T-tubule remodeling and Ca^{2+} handling dysfunction in response to pressure overload is dependent on protein kinase C likely by modifying actin polymerization / depolymerization dynamics.[14] Cumulatively, these findings point to the strong functional interaction that likely exists between cytoskeletal actin and T-tubule structural integrity whereby the actin cytoskeleton shapes and maintains the T-tubule network in cardiomyocytes. It will also be of interest to determine whether NEXN has roles beyond T-tubule/JMC formation. Furthermore, are there other proteins involved with NEXN in maintaining the T-tubule organization? Answering these questions will further enhance our understanding of cardiac E-C coupling regulation in health and dysregulation in heart disease. Such studies would not only define the role of NEXN in T-tubule remodeling in heart failure but would also indicate whether NEXN should be pursued as a novel therapeutic target to preserve the stability of T-tubule system in the treatment of heart failure.

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