EDITORIAL COMMENT

Nebulette Mutations in Cardiac Remodeling

Big Effects From a Small Mechanosensor*

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Within the cytoskeletal framework of the heart, sarcomeres form the contractile machinery of cardiac muscle. Proteins associated with the thick and thin filaments and the Z-disk participate in maintenance of the sarcomere structure. The Z-disks connect with integrins and dystroglycan of the sarcolema, allowing transmission of force generated by myofilaments to the adjacent sarcomeres, the extracellular matrix, and ultimately, to other cells. Additionally, proteins shuttling between sarcomeric and nonsarcomeric locales convey signals from the contractile machinery to the nucleus (1). These highly coordinated and overlapping functions of the cytoskeletal machinery enable rapid adjustments in cardiac muscle to changes in physiological requirements. Mutations of several proteins associated with sarcomere, Z-disk, and cytoskeletal assembly and function cause dilated cardiomyopathy (DCM) and heart failure (2). Considering the important role of the Z-disk in cardiac homeostasis, it is not surprising that mutations in Z-disk proteins not only alter its physical connection with myofilaments and cytoskeletal proteins, but also affect the generation and transmission of mechanical and biochemical signals.

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Nebulette, the cardiac isoform of the giant (>600 kDa) actin-binding protein nebulin (found in skeletal muscle), is localized to, and predicted to extend <150 nm beyond, the Z-disk. Nebulette consists of a short N-terminal domain and 22 nebulin-like repeats that are connected to a C-terminal SH3 domain by a short linker domain (3). Overexpression of the linker or SH3 domains in chicken embryonic cardiomyocytes resulted in a loss of endogenous nebuline and a decrease in filament length (4,5), suggesting a role for nebuline in both thin filament and Z-disk assembly, function, and stability (3,5,6). Nebulette interacts with a large number of thin filament-associated and Z-disk–associated proteins (3). The central repeat domains associate with actin, troponin, and tropomyosin; the SH3 domain interacts with actin, α-actinin (7), CapZ, titin, myopalladin, zyxin (8), desmin (9), and filamin C (10).

In this issue of the Journal, Purevjav et al. (11) have described de novo nebuline mutations in patients with DCM and endocardial fibroelastosis (EFE). The data in part support the authors’ prior proposal that genes encoding cytoskeletal and sarcomeric proteins represent a major component of the “final common pathway” in the development of heart failure (12). Previously, nebuline polymorphisms have been linked with nonfamilial DCM (13); this study identifies 4 individual, de novo mutant variants of the human nebuline gene (1, K60N, had been previously reported in dbSNP; rs41277374). Two of the 4 mutations reside in the actin-binding domain (K60N, Q128R); the other 2 are located within the nebulin-like repeat region (G202R, A592E). Importantly, human cardiac disease phenotypes were recapitulated in cardiac-targeted transgenic mouse models: cardiac expression of K60N or Q128R mutations resulted in embryonic lethality, whereas the other 2 (G202R, A592E) led to progressive DCM. This confirmatory in vivo transgenic mouse data revealed distinct cytoskeletal changes and myofibrillar organization resulting from various nebuline mutations, and suggests roles of nebuline in early cardiac development. Finally, the effect of nebuline mutations on nebuline subcellular distribution and the effect of cyclical mechanical stretch was assessed in embryonic rat cardiomyocytes.

In the present study (11), the authors identify multiple, isolated mutations in a single nebuline gene, revealing distinct Z-disk genotypes affiliated with distinct DCM phenotypes in both humans and transgenic mice. With the recent identification of numerous Z-disk mutations, genotype/phenotype analyses such as those outlined in the current study may help further identify, validate, and/or stratify genetic subsets of DCM patients, possibly extending the screening benefit to the patient’s immediate and extended families. The divergence in DCM phenotype across the 4 identified nebuline mutations (functionally validated in cardiac transgenic mice) reflects the multiple roles of nebuline in modulating cardiomyocyte stretch-strain with distinct structural/cytoskeletal modifications.

Each region of nebuline appears to be critical for myofibrillar assembly and stability. Functional mutations within the nebulin-like repeat region (such as of the G202R and A592E mutations described by the authors) affect maintenance and stabilization of the Z-disk assembly (14).
Defective force generation and force transmission are widely proposed pathophysiological models of DCM (15). The cytoskeleton is a dynamic, adaptive structure that physically links the contractile machinery to the extracellular matrix. While changes in sarcomere/calcium handling proteins can affect actin-myosin interactions (16) and force generation such as in the G202/Q128R mice, alterations in cytoskeletal proteins (A592E mice) reduce force transmission efficiency (17). Given the unique properties of nebulette’s interaction with both cytoskeletal and sarcomeric proteins across different domains, loss of coordinated interaction between the sarcomere, cytoskeleton, and the sarcolemma may result in reduced cardiac contractile function, ultimately leading to pathologic cardiac hypertrophy and cardiomyopathy.

As previously described, the Z-disk, well recognized as a specialized stretch-interpreting sensor (18), enables the cardiomyocyte to sense increased mechanical load and respond with multiple changes, including altered gene expression, resulting in pathologic hypertrophy that compensates into heart failure. Nebulette consistently associates with the Z-disk assembly, persisting from genesis of the myofibril of developing cardiac muscle (7) to its final position at the Z-disk in the mature heart. The authors (11) highlight that, during cyclic mechanical strain, nebullette is initially localized to the perinuclear region, followed by its distribution along F-actin filaments reaching the cell periphery. In contrast, mutant nebullette proteins preferentially localized to the perinuclear region, with delayed expression of this mutant along maturing actin filaments. Further, in the Q128R mutants, nebullette appeared to be dissociated from Z-disks, accompanied by a loss of desmin.

Calcineurin and NFAT are emerging candidate messengers responsible for transmission of stretch signals from Z-disks to the nucleus (18). Mechanical stretch can lead to activation of multiple stretch-activated kinase cascades, as well as changes in the activity of various ion channels/exchangers, presumably all as a result of an acute hypertrophic process. Desmin, a major cytoarchitectural protein also residing at both the Z-disk and intercalated disk, is known to play a vital role in transmitting longitudinal stretch signals (19). Nebulette, in its position at the nexus of sarcomeric assembly, may play dual roles as both a structural protein at the Z-disk, and also as a regulatory protein (possibly through its binding partners) of signaling pathways between both the nucleus and the Z-disk during cardiac development and mechanical stretch. Although both interesting and informative, experiments in H9C2 cells subjected to 2-dimensional strain may partially limit extrapolation of the findings to an intact, 3-dimensional beating heart. Characterizing the timing of key events in assembly of the nebullette interactome during development, perhaps using real-time microscopy with the various mutants, will be worthy of further investigation. Additionally, studies should also aim to determine whether the stretch sensing, signal-

In summary, this exciting study by Purevjav et al. (11) identifies nebullette mutations associated with a continuum of cardiomyopathy phenotypes and severities in humans. Importantly, the study validates the functional effects of the newly identified nebullette mutants in vivo using cardiac transgenic mice, revealing an array of pathologic cardiac phenotypes from neonate to adult. This finding suggests that nebullette is required for normal genesis of the sarcomere and stabilization of the Z-disk during both development and adult cardiac contractility. Further, the study identifies nebullette as an essential component of the stretch sensor machinery contributing to the pathophysiology of DCM and EFE, and opens the door to studying the roles of domain-specific Z-disk interactions affecting mechanical and biochemical pathways for all cardiomyopathy phenotypes.

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