Unraveling Enigma in the Z-Disk

Xuejun Wang, Huabo Su

The Z-disk, a fascinating structure in myofibrils, demarcates sarcomeres and serves as not only a mechanical focal point but also a likely signaling nexus in mediating and regulating the functions of striated muscle. In the Z-disk, the barbed end of actin filaments and the titin filaments from the 2 opposing sarcomeres are cross-linked primarily by α-actinin, allowing transmission of tension between sarcomeres during contraction. At the Z-disk level, myofibrils are registered laterally to one another, to the cell membrane, and to the nuclear envelope by a network of desmin-containing intermediate filaments. Additionally, the Z-disk may forge linkages with integrin and dystrophin-glycoprotein complexes through interactions with proteins such as filamin C. Therefore, the Z-disk is the focal point in myofibrils that arguably best communicates with both intramyofibrillar and extramyofibrillar cytoskeletons, with physical linkages to the cell membrane where both inside-out and outside-in signaling occur, and to the nucleus where gene expression takes place. Notably, mutations of many genes encoding Z-disk constituents and associated proteins have been linked to myofibrillar myopathies. Hence, these proteins have attracted a broad interest for a better understanding of the structure and genesis of myofibrils, signaling events and protein homeostasis in cardiomyocytes, and the function of the heart.

It becomes increasingly tempting to believe that the Z-disk is not simply a mechanical joint responsible for force reception, transduction, and transmission, but also functions to sense mechanical stress and strain, and subsequently initiate signaling pathways to alter gene expression in response to this stress. In most cases, Z-disk proteins function as scaffolds to bring the signaling effectors to the substrates, thereby facilitating their interaction. An increasing number of signaling proteins including protein kinases and phosphatases interact with Z-disk proteins and are concentrated at the Z-disk, which may help confine chemical signals to a specific subcellular location, enhance the efficiency of substrate-enzyme interactions, and reduce nonspecific effects.

Enigma proteins, including at least 3 known members (enigma, enigma homolog [ENH], and Cypher [mouse]/ZASP [human]) are a subfamily of Z-disk proteins that belong to PDZ-LIM (acronym of PSD 95, DLG, ZO-1, and LIN-11, Isi-1, MEC-3, respectively) proteins. Typical Enigma proteins contain 1 PDZ domain and 3 LIM domains. Global or cardiomyocyte-restricted ablation of the Cypher gene in mice has been shown to cause dilated cardiomyopathy and premature death, with specific activation of extracellular signal-regulated kinase and Stat3 signaling pathways in Cypher-deficient hearts. Moreover, mutations in ZASP have been linked to various forms of cardiomyopathy and myofibrillar myopathy in humans. Compared to Cypher, much less is known about the role of the other 2 Enigma proteins, enigma and ENH, in the heart.

In this issue of Circulation Research, a study by Cheng et al begins to investigate the physiological and pathophysiologic significance of ENH in the heart using global and conditional gene targeting in mice. In humans, the ENH gene gives rise to 4 splicing variants. The LIM domains-containing long isoform (ENH1) is ubiquitously expressed, whereas the expression of the short isoforms (ENH2–4) is limited to cardiac and/or skeletal muscles. A recent study reveals differential expression of Enh splice variants during heart development and their seemingly distinct roles in mediating cardiomyocyte hypertrophy in rodents. Specifically, Enh1 is highly expressed in the fetal and neonatal hearts, downregulated in adult hearts but upregulated during pressure overload hypertrophy. Enh4 expression appears to show the opposite pattern. Overexpression of Enh1 stimulates, but overexpression of Enh4 suppresses, neonatal rat cardiomyocyte hypertrophy in culture. Using RT-PCR and sequencing analysis, Cheng et al identified 5 additional Enh splice variants in mouse hearts, including Enh1b, -1c, -1d, -1e (original Enh1 is renamed as Enh1a) and Enh3a (original Enh3 is renamed as Enh3b). Given the sequence similarity of the newly identified Enh1 isoforms (Enh1b, -1c, -1d, -1e) to Enh1a, one may speculate that these isoforms may be functionally redundant but this remains to be tested. Exon 3 of Enh, encoding a portion of the PDZ domain, is shared by all splice variants in mouse hearts. Chang et al adopted a gene targeting strategy that deletes exon 3 of the Enh gene. Therefore, the Enh knockout mice lose expression of all Enh isoforms and thereby are suitable to study the combined consequence of loss-of-function of all Enh isoforms.

Consistent with a structural role of Enh in the Z-disk, electron microscopic analysis shows apparent Z-line abnormalities in Enh-deficient cardiomyocytes, although embryonic development of the heart does not appear to be affected by Enh deficiency. Both global and cardiac-specific Enh knockout mouse models survive through embryonic development, but these mice develop dilated cardiomyopathy under standard conditions in a mouse facility. The phenotype appeared to be less severe than what was observed in Cypher-null mice as no increased fatality was observed at least during the early postnatal months. The milder phenotype
of both global and cardiac-specific ablation of Enh than what was observed in the Cypher knockout may have something to do with a partial compensation from Cypher long form (CypherL) and its interacting partners because CypherL and myotilin are significantly upregulated in the Enh-deficient heart. Compared with wild type controls, Enh-null mice showed a blunted increase in the left ventricular wall thickness, more severe chamber dilatation, and more pronounced functional deficit in response to pressure overload induced by transverse aortic constriction. These findings may suggest that loss of Enh in the Z-disk impairs the mechanical stress sensing apparatus and leads to a failure to activate the signaling pathways that are required for initiating a compensatory response to the increased mechanical stress. PDZ-LIM proteins in the Z-disk serve as adaptor proteins. The PDZ domain mediates the interactions with the cytoskeleton, whereas the LIM domain and/or additional internal domains recruit signaling proteins to the Z-disk. Previous studies have identified protein kinase (PKCε) isofrom (PKCε) and PKD1 as the binding partners of ENH.13,14 Interestingly, it appears that neither PKCε nor PKD1 are disturbed in Enh-null hearts.

A very interesting new finding by Cheng et al is that Enh deficiency causes progressive and specific decreases in the protein level of Cypher short form (CypherS) and Calsarcin 1, which is exacerbated by transverse aortic constriction.15 The decreases of Cypher and Calsarcin 1 protein levels were not accompanied by a reduction in their transcript levels. Further in vitro assays show that Enh, CypherS, and Calsarcin 1 interact with each other. These findings lead to the conclusion that Enh, CypherS and Calsarcin 1 form a protein complex in the Z-disk, and loss of Enh disrupts this complex and thereby destabilizes its constituent proteins.16 Calsarcin 1 was originally identified as a sarcomeric protein that tethers calcineurin to the Z-disk.10 The loss-of-function study suggests that Calsarcin 1 inhibits the calcineurin–NFAT (nuclear factor of activated T cells) pathway, but Calsarcin 1–null mice do not show remarkable phenotypes under baseline physiological conditions.20 Consistently, the downregulation of Calsarcin 1 in Enh-deficient hearts does not appear to alter the activity of the calcineurin–NFAT pathway, as evidenced by conservation of the nuclear translocation of NFATc4 and the expression of a NFAT target gene MCIP (modulatory calcineurin-interacting protein) between Enh-null and wild-type mouse hearts.16 However, it remains unclear whether the downregulation of Calsarcin 1 in Enh-null mice sensitizes the calcineurin–NFAT pathway when the heart is mechanically challenged. This is of particular interest because mutations in the gene encoding Calsarcin 1 (myozenin 2 [MYOZ2]) have been linked to human familial hypertrophic cardiomyopathy, but molecular mechanisms by which the mutations lead to cardiac hypertrophy remain to be delineated.21

With the discovery of new Enh splice variants in mouse hearts, generation of global and conditional Enh knockout mouse models, identification of the Enh-CypherS-Calsarcin 1 protein complex, and revealing the interdependency of the protein stability among these proteins, the study by Cheng et al16 provides important contributions to the research efforts aiming at unraveling the (patho)physiological significance of the Enigma subfamily in the heart.

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Disclosures

None.

References


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