JCI The Journal of Clinical Investigation

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J Clin Invest. 2018;128(12):5219-5221. https://doi.org/10.1172/JCI124778.

Commentary

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STAT3: a link between CaMKII–β_{IV}-spectrin and maladaptive remodeling?

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Spectrins in the cardiomyocyte cytoskeleton

Cardiac function requires tightly regulated signaling from the extracellular space to the cytoplasm across the plasma membrane and from cell to cell across the intercalated disc. Efficient communication is achieved through organized ion channel hubs known as microdomains that facilitate spatially and temporally accurate signaling. These microdomains are largely maintained by the cardiomyocyte cytoskeleton, which not only bolsters cardiomyocyte structure but also acts as a scaffold for ion channels and signaling molecules. Spectrins are cytoskeletal proteins with α and β isoforms that heterodimerize and bind the actin cytoskeleton to a variety of membrane proteins (1). In cardiomyocytes, α_{II}-spectrin dimerizes with β_{II}-spectrin at the plasma membrane and sarcoplasmic reticulum (2) and with β_{IV}-spectrin at the intercalated discs (3). Through binding to adapter proteins known as ankyrins, spectrins organize several key ion channels including NCX, Na/K ATP-ase, $Ca_v1.3$ to T-tubules, IP_3R and RyR2 to the sarcoplasmic reticulum, and $Na_v1.5$ to intercalated discs (4).

In their physiological role, spectrins are required for maintenance of the cardiomyocyte ultrastructure and contractile function. β_{II}-Spectrin conditional KO mice develop cardiac dysfunction (5). Additionally, spontaneous murine mutations arising in β_{IV}-spectrin known as quivering (*qv*) mice have revealed a critical role of β_{IV}-spectrins (6). For instance, the qv^{4J} mutation disrupts the interaction between β_{IV}-spectrin and ankyrin-G, thereby mislocalizing the two-pore potassium channel TREK1, which causes cardiac arrhythmias in mice (7). Likewise, qv^{3J} mice lacking the C-terminal CaMKII–β_{IV}-spectrin interaction develop disorganized and dysfunctional membranes in neuronal, pancreatic (8), and cardiac muscle cells (3). These findings demonstrate the important physiological role of cardiomyocyte ultrastructure maintenance by β-spectrins.

▶ **Related Article: p. 5561**

Conflict of interest: XHTW is a founding partner of Elex Biotech, a start-up company that develops drug molecules targeting ryanodine receptors for the treatment of cardiac arrhythmia disorders.
Reference information: *J Clin Invest*. 2018;128(12):5219–5221. https://doi.org/10.1172/JCI124778.

In addition to their physiological roles, β-spectrins may also contribute to pathological changes during pressure overload that precipitate detrimental cardiac remodeling. For instance, β_{II}-spectrin is cleaved by calpain-II during heart failure (5). However, it remains uncertain whether the cleavage products further contribute to maladaptive cardiac remodeling in addition to the degradation of β_{II}-spectrin. Previous studies revealed that β_{IV}-spectrin is also decreased in human heart failure (7); however, the role of β_{IV}-spectrin in heart failure is yet to be completely understood.

Spectrins modulate signaling

One of the major functions of spectrins is to act as a hub for signaling molecules. Kinases, phosphatases, transcription factors, and other signaling molecules localize to subcellular domains containing spectrins (3). Previous studies have shown that an imbalance in kinase and phosphatase activity can modulate ion channel activity and promote arrhythmias and heart failure (9). In addition to modulating ion channels, kinases and phosphatases that localize to the spectrin scaffold can modulate β-spectrins themselves. Changes in the phosphorylation state of β-spectrins can modulate membrane stability. For instance, enhanced β-spectrin phosphorylation disrupts membrane stability during cellular remodeling events such as mitosis (10). The consequences and downstream mechanisms of β-spectrin phosphorylation need further exploration in the context of cardiomyocyte membrane stability and heart failure progression.

β-Spectrins also sequester other signaling molecules including transcription factors, such as SMAD3 (11) and YAP (12), and prevent their nuclear localization. In addition to physical sequestration, transcription factors are regulated by phosphorylation status. The JAK/STAT pathway is thought to contribute to increased inflammation in addition to cardiomyocyte hypertrophy.

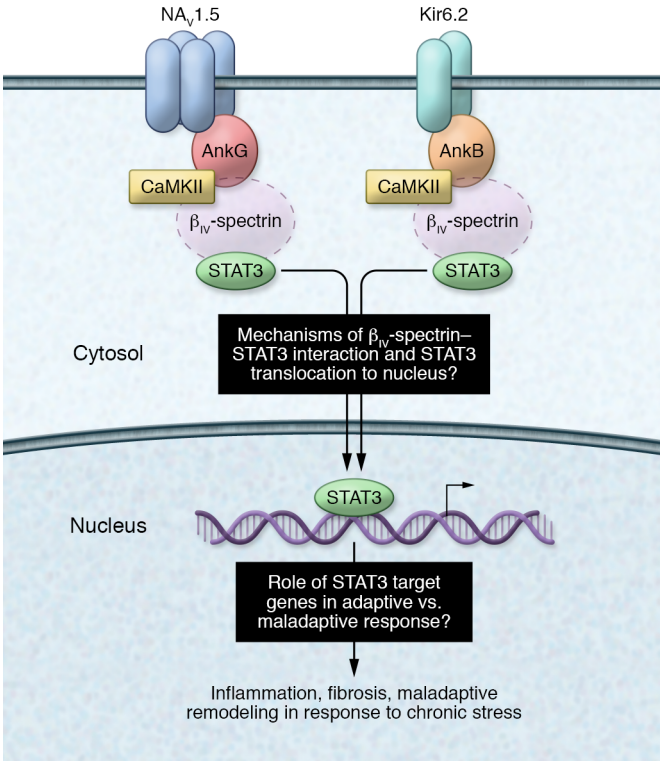

Figure 1. Schematic of the CaMKII/β_{IV}-spectrin signaling axis at the cell membrane. CaMKII and β_{IV}-spectrin bind to ankyrin-G (AnkG) and multiple ion channels such as Kir6.2 and $Na_v1.5$ in the plasma membrane. In the present study, Unudurthi et al. show that, upon being phosphorylated by CaMKII, β_{IV}-spectrin is degraded, which leads to STAT3 release and translocation into the nucleus, activating gene transcription in response to chronic stress. Some of the unanswered questions for future studies are highlighted in the black boxes in the figure.

Canonically, STAT3 is activated by JAK phosphorylation upon cytokine or angiotensin signaling at the plasma membrane and translocates to the nucleus, where it acts as a transcription factor and cofactor (13). Although STAT3 nuclear translocation has been shown to promote maladaptive remodeling including hypertrophy (13) and fibrosis, complete STAT3 KO in cardiomyocytes actually increases inflammation and impairs cardiac contractility (14). Moreover, STAT3 may also promote cardiomyocyte survival (15). These paradoxical differences may be due to differences in canonical versus noncanonical STAT3 signaling pathways, which are currently under investigation (16). Thus, STAT3 signaling is complex and will require further investigation to tease apart the adaptive and maladaptive responses to its activation.

Spectrins in chronic pressure overload

In the accompanying article in this issue of the *JCI*, Unudurthi et al. (17) demonstrate a novel CaMKII/β_{IV}-spectrin/STAT3 pathway that is activated in response to chronic stress (Figure 1). This study is based on a previous finding by the authors showing that CaMKII and β_{IV}-spectrin interact with each other at the intercalated discs (3). In this study, the authors show that β_{IV}-spectrin binds to STAT3 under basal conditions and sequesters it, forming a "statosome" at the intercalated discs. Under chronic pressure overload, CaMKII-mediated phosphorylation leads to β_{IV}-spectrin degradation, resulting in the release of β_{IV}-spectrin and STAT3 from the membrane. STAT3 subsequently translocates into the nucleus and activates the transcription of target genes, resulting in fibrosis, inflammation, maladaptive remodeling, and, eventually, decreased cardiac function.

The authors used the qv^{3J} mouse model lacking the CaMKII-binding site on β_{IV}-spectrin, thus inhibiting CaMKII–β_{IV}-spectrin interaction completely. Therefore, these mice are an ideal model for studying the role of CaMKII–β_{IV}-spectrin interaction in pressure overload–induced remodeling and heart failure. However, the abolished CaMKII–β_{IV}-spectrin interaction limits the localization of CaMKII to the membrane and inhibits its ability to phosphorylate other target proteins such as Kir6.2 (8) and $Na_v1.5$ (3). Moreover, the protection afforded by the qv^{3J} mutation is similar to that in total CaMKII-KO mice (18). Since CaMKII is known to be involved in multiple signaling pathways, a better understanding of the role of downstream proteins other than β_{IV}-spectrin in CaMKII-mediated adverse remodeling is necessary.

Future considerations

Likewise, the specific molecular mechanisms of how CaMKII–β_{IV}-spectrin result in an altered "statosome" and dysregulation of STAT3-mediated gene transcription are not known. Elaborate studies need to be planned to uncover the determinants of β_{IV}-spectrin–STAT3 interaction and how STAT3 translocates into the nucleus upon β_{IV}-spectrin degradation. In the canonical pathway, STAT3 needs to be phosphorylated at Y705 and S727 to translocate into the nucleus (16). On the other hand, noncanonical pathways are being identified, in which STAT3 remains unphosphorylated (USTAT3) (16). This study did not consider the STAT3 phosphorylation status, but in the future it will be important to distinguish whether STAT3 is phosphorylated at Y705 and/or S727 either directly by CaMKII or indirectly through another kinase. An in-depth understanding of the molecular mechanisms of STAT3 translocation will be a critical stepping stone to developing β_{IV}-spectrin and STAT3 as therapeutic targets (Figure 1). Moreover, contradictory data demonstrate that STAT3 may be either beneficial or harmful in the context of cardiac hypertrophy and heart failure (19). To make things more complicated, STAT3 is known to have nongenomic roles in microtubule stabilization (with stathmin) (20) and mitochondrial function (with GRIM19) (21). Therefore, targeting STAT3 therapeutically may not be a straightforward strategy. Studying the role of multiple STAT3 target genes that were found to be differentially expressed upon chronic pressure overload in the qv^{3J} and WT mice could be the answer to this conundrum. It is possible that the beneficial as well as harmful effects of STAT3

are mediated by differential expression of genes carrying out these "good" (adaptive) or "bad" (maladaptive) phenotypes (Figure 1). Further study of these genes could reveal some of the "bad genes" as potential therapeutic targets.

Pathways downstream of the CaMKII/β_{IV}-spectrin signaling axis independent of STAT3 also need to be explored. CaMKII regulates other transcriptional regulators such as HDAC and NFAT, which are known to regulate the transcription of multiple genes. Therefore, while studying the organization and function of "local" CaMKII/β_{IV}-spectrin signaling domains, the findings from this study need to be correlated with the global response of the cell to chronic stress. Investigating the interplay between multiple molecular pathways and understanding how a particular pathway is selected over others in response to chronic stress should be an important step toward the development of therapeutic strategies.

Acknowledgments

XHTW is supported by grants from the NIH (R01-HL089598, R01-HL091947, R01-HL117641, and R41-HL129570); a grant from the Saving Tiny Hearts Foundation; and the Quigley Endowed Chair.

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