Mending a Broken Heart: The Role of Sarcospan in Duchenne Muscular Dystrophy–Associated Cardiomyopathy

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Duchenne muscular dystrophy (DMD) is the most severe and most common form of X-linked muscular diseases. It has been known for over 25 years that DMD is caused by a gene mutation leading to the near total absence of functional dystrophin protein. Transcription of the gene is controlled by 3 independent promoters of which the muscle (M) promoter drives high expression of dystrophin in skeletal and cardiac muscle.[1] Dystrophin is associated with a cytoskeletal lattice commonly referred to as a costamere. It has been shown that dystrophin, through the membrane-spanning region, physically links the extracellular matrix to the actin cytoskeleton.[2] The costameres physically couple the sarcolemmal membrane with the Z disk region and as a result, dystrophin stabilizes the sarcolemma against the mechanical forces produced during the contraction process.[3] The importance of dystrophin in the structural support of the sarcolemma has been clearly shown in data from the *mdx* mouse, a model of DMD. In this mouse, dystrophin and/or select dystrophin domains have been deleted and the resulting striated muscle cells demonstrate markedly increased fragility, disorganization of the costameres, and necrosis.[3] This knowledge led to the "mechanical hypothesis" in which the lack of dystrophin leads to increased membrane fragility and the progressive cellular necrosis characteristic of the disease. Studies published in the 1990s using cultured myotubes and isolated mature muscle fibers showed that DMD and *mdx* myotubes were more susceptible to hypo-osmotic shock than control cells, indicating that *mdx*-derived cells were fragile and prone to mechanical injury.[4,5] Interestingly, the membrane fragility/mechanical injury hypothesis had first been hypothesized in the 1980s in experimental model systems as the underlying mechanism of ischemia/reperfusion injury as well as the calcium paradox.[6,7] Subsequent groups confirmed that inhibition of contraction at the onset of reperfusion prevented and/or substantially reduced cell death in experimental systems.

At the subsarcolemmal location, dystrophin is incorporated into a larger complex of proteins known as the dystrophin-associated protein complex containing dystrophin, dystroglycans, sarcoglycans, sarcospan, dystrobrevins, and syntrophin, which is commonly referred to as the dystroglycan complex (DGC).[1] In addition to its role in structural support of the sarcolemmal membrane, the DGC has been proposed to constitute a potential cellular signaling complex by virtue of the scaffolding nature of dystrophin and the association of many putative cellular signaling molecules into the DGC, raising the possibility that the DGC may be capable of transmitting extracellular-mediated signals to the inside of the cell through the cytoskeleton. Indeed, β-dystroglycan has been shown to interact with mitogen activated protein kinase (MEK) and extracellular signal-regulated kinase (ERK), suggesting that dystroglycan can influence the ERK-mitogen activated protein kinase (MAPK) cascade through such a scaffolding interaction.[8] Laminin also interacts with dystroglycan and is capable of recruiting signaling complexes (eg, Grg2-Sos1) to dystroglycan.[9] Recent experimental studies from Vander Heide have shown that cytoskeletal-based signaling is important in cellular protection in cardiac ischemia–reperfusion injury.[10] These studies imply that the necrosis characteristic of DMD may be in part due to interruption and/or quenching of important cell survival signals involving the proteins present in the DGC complex.

The sarcoglycan complex is composed of 4 isoforms (α, β, γ, δ) and sarcospan (SSPN). SSPN consists of 4 membrane-spanning segments, while sarcoglycans contain 1 membrane-spanning region.[3,11] It has been suggested that the function of the sarcoglycan complex is to strengthen the interaction of β-dystroglycan with α-dystroglycan and dystrophin.[12] Mutations in any of the 4 transmembrane proteins result in autosomal recessive limb-girdle muscular dystrophy.[1,11] Several studies on sarcoglycans have yielded clues that they may also play an important role in intracellular signal transduction.[13] In addition,

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lack of δ–sarcoglycan has been shown to reduce neuronal nitric oxide synthase levels as well as displacing it from the sarcolemma, potentially resulting in loss of vasodilatory functions in the smooth muscle cells of vascular structures.[14] Crosbie-Watson has shown that SSPN improves cell surface expression at 3 important major laminin-binding complexes: the DGC, the utrophin–glycoprotein complex, and at 7β1-integrin.[15] Since utrophin–glycoprotein is homologous to the DGC and α7β1-integrin is the major integrin expressed in adult skeletal muscle, utrophin–glycoprotein and α7β1-integrin have been proposed as compensatory complexes for the loss of the DGC complex typically seen in DMD.[11] Furthermore, it has been shown that transgenic expression of SSPN causes increased levels of dystrophin at the sarcolemma in normal skeletal muscle and increased levels of utrophin–glycoprotein and α7β1D integrin at the sarcolemma, which restored laminin-binding and ameliorated the skeletal muscle necrosis.[13,16]

In this issue of *JAHA*, Parvatiyar et al [17] extend this interesting story into cardiac muscle to determine whether the same mechanisms may underlie the cardiac dysfunction characteristic of DMD. They investigated baseline structural and functional consequences of SSPN ablation in mouse cardiac muscle and also determined whether transgenic overexpression of SSPN in the *mdx* mouse model would ameliorate the cardiac symptoms. The results show that mice lacking SSPN exhibited reduction of dystrophin levels, decreased sarcoglycan and integrin binding to the DGC, and reduction in laminin binding. Furthermore, SSPN-null mice showed cardiac enlargement, increased myocyte hypertrophy, and increased interstitial fibrosis in response to an acute β–adrenergic challenge. The authors interpret the results to indicate that SSPN plays an important role in the regulation of sarcolemmal expression of laminin-binding complexes. However, it is also apparent that loss of SSPN and the associated changes in laminin complex binding have effects on baseline cardiac structure and the response to acute adrenergic stress. The cause of the increased fibrosis in the SSPN-null mice is not clear but could be due to either vasospasm secondary to loss of δ–sarcoglycan in the vasculature or alternatively, due to transient episodes of ischemia secondary to adrenergic stimulation superimposed on mechanically fragile cells.

Transgenic overexpression of human SSPN in the DMD (*mdx*^TG) mouse improved adhesion, sarcolemmal structure, and cardiac function supporting a critical role for SSPN in protecting against both transient and chronic myocardial injury. Although these data are provocative, the results warrant further investigation. The hypertrophy measured in the SSPN-null mice in response to isoproterenol is due to activation of hypertrophic signaling pathways. However, the hypertrophic response is likely complex and may involve activation of multiple signaling pathways. Secondly, the authors provide convincing data that the reduction in cell death/increased sarcolemmal stability is due at least in part to compensation provided by the increased expression of utrophin and β1D integrin, transmembrane proteins involved with extracellular matrix attachment. However, as discussed above, there is ample and intriguing evidence that cytoskeletal-based signaling can activate survival pathways, thereby reducing cell death. Given that DGC contains many signaling proteins, it is possible that at least a component of the increased cell fragility/cell death seen in the SSPN-null mice, *mdx* mice, and even DMD may be due to interruption of critical cytoskeletal-dependent cell survival signaling. The role of SSPN in cardiac muscle is nascent and likely to be complex. The authors cite a Genome Wide Association Study (GWAS) study that shows polymorphisms in the SSPN locus 12p.11.2 are positively correlated with increased left ventricular mass in several families, raising the possibility that SSPN variants may affect other cardiomyopathies related to mutations in other cytoskeletal and/or contractile genes. It is also possible that SSPN variants play a role in cardiac disease resulting from mutations in proteins associated with the DGC/integrins or even heart failure resulting from acquired myocardial diseases such as ischemic heart disease. Such possibilities are intriguing and deserve more investigation.

The cardiac disease in DMD is progressive and leads to ventricular dysfunction, dilatation, and failure. Pathologic changes described in autopsied human hearts are nonspecific and similar to those described in the SSPN-null mice and include mixtures of myocyte hypertrophy, atrophy, and fibrosis. The only current therapy regimens consist of corticosteroids; however, more than 25% of DMD patients are not treated with corticosteroids due to intolerance of the side effects or lack of response.[18] Clearly there is need for more effective therapies to treat DMD. The unique biochemical properties of SSPN have made it an attractive target for muscular dystrophies. Early preclinical and clinical trials indicate that gene therapy via injection of recombinant adeno-associated viral vectors may be a viable approach, at least for skeletal muscle disease.[19] However, it is less clear whether such an approach would be viable for treatment of the respiratory failure (diaphragm) and/or cardiac failure often associated with the fatal consequences of DMD. The study by Parvatiyar et al has provided new and provocative results indicating that alternative therapies for DMD and the other muscular dystrophies are possible through selective manipulation of cytoskeletal proteins and encourages more research into the area of targeted gene therapy.

Disclosures

None.

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