Repairs the myofilaments to heal the heart

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FAMILIAL HYPERTROPHIC CARDIOMYOPATHY (FHC) is a genetic disease caused by autosomal dominant mutations in the genes that encode sarcomeric proteins. Hundreds of FHC disease-causing mutations have been identified (reviewed in Ref. 14). These mutations are predominantly although not exclusively missense mutations. In vivo and in vitro studies support the notion that FHC alleles produce mutant proteins that incorporate into the myofilament. The abnormal proteins exert dominant-negative effects, particularly thin filament proteins like α-tropomyosin (TM), although gain-in-function effects may have a role in disease caused by myosin heavy chain mutants (14). The cellular and molecular events triggered by the causal mutations have not been fully elucidated. Specifically, it remains to be determined how a defect in myofilament function results in a complex cellular, myocardial, and disease phenotype.

In this issue of the American Journal of Physiology-Heart and Circulatory Physiology, Jagatheesan et al. (8) investigate reversal of a myofilament Ca2+-sensitivity defect produced by a FHC-associated mutation of α-TM. The α-TM glutamic acid-to-glycine mutation at residue 180 (Glu180Gly) described in this article is of particular interest for several reasons. Although thin filament mutations account for a relatively low percentage of FHC mutations in a population presenting for clinical testing, those affected by this mutation have relatively early onset of disease and sudden cardiac death (15–17). This mutation occurs in a key domain of TM involved in stabilizing troponin (Tn) to the thin filament via binding of TM to TnT. While animal models of this mutant have been variable, perhaps because of differences in genetic background, the transgenic α-TM Glu180Gly line described by this group has been shown previously to mimic many aspects of the human phenotype, including increased Ca2+-sensitivity of the myofilaments, diastolic dysfunction, cardiac hypertrophy, myocardial fibrosis, and early cardiac demise (11). The finding of a progressive and deadly phenotype makes this an attractive model to test reparative therapies.

This report enhances our understanding of cellular and molecular events that lead to hypertrophic remodeling and fibrosis. The authors created a double-transgenic mouse model by crossing the α-TM Glu180Gly transgenic with a line expressing a chimeric TM containing an α-TM amino terminus and the carboxy terminus of β-TM, the cardiac fetal isoform. This line was previously demonstrated to produce myofilaments with decreased sensitivity to Ca2+ (9). The double-transgenic mice created by crossing these lines not only displayed normalized myofilament Ca2+-sensitivity; most importantly, the mice exhibited a normal organ-level phenotype with no pathological abnormalities and apparent normalization of the life span of the double-transgenic mice. These findings indicate that the abnormal myofilament function produced by the α-TM Glu180Gly mutant plays a central role in signaling the subsequent development of cardiac hypertrophy and fibrosis.

Role of Tropomyosin in Contractile Regulation

Contraction of muscle results from the highly coordinated Ca2+-dependent interactions between the myofilament proteins. The thin filament is comprised of filamentous actin, TM, and Tn. TM is an alpha helical coiled coil protein that exists as a dimer and is arrayed in a head-to-tail formation stabilizing filamentous actin (18). When Ca2+ is released from the sarcoplasmic reticulum during systole, it binds to the low-affinity regulatory site on cardiac TnC. However, it is the subsequent switching of binding of TnI domains from actin-TM to TnC in the presence of Ca2+ that promotes the interaction of actin with myosin and thus contraction. Although allosteric protein-protein interactions are involved, TM is central in maintaining relaxation in diastole via steric hindrance of cross-bridge binding to the thin filament and removal of that hindrance by movement of TM on the filament in the presence of thin filaments, myosin heads, and systolic levels of Ca2+. This has been well established by recent structural models (5).

The mutations identified in α-TM that lead to FHC generally fall within the discrete region of the amino-terminal domain or, like this mutation, a more central domain that includes the TnT binding site. The Glu180Gly α-TM mutation produces a charged-to-neutral amino acid substitution. The interaction between the TM and TnT may be altered by this mutation, and in addition it may produce disruption of the normal TM-actin binding due to the amino acid charge difference in a molecule that is highly dependent on the combination of polar and hydrophobic interactions. In vitro studies of the Glu180Gly mutant suggest that it has decreased affinity for actin, which may destabilize TM structure, and there may also be a decreased ability to inhibit actomyosin ATPase (2, 3, 10). However, the precise mechanism of how the chimeric α-/β-TM repairs the myofilament is not clear. Although correction of the steady-state force-Ca2+-relationship occurs, this is likely a readout for stabilizing TM structurally, increasing the inhibitory capacity of TM in the myofilament, and/or correction of the kinetics of the contractile process.

Therapies for Hypertrophic Cardiomyopathy

Ca2+ is critical for normal muscle contraction and relaxation as well as an important signaling molecule in the heart. There is strong evidence that abnormalities in Ca2+ cycling contribute to the pathophysiology of cardiac dysfunction (1, 7). Previous work has demonstrated that Ca2+ cycling is likely disturbed in this α-TM Glu180Gly transgenic mouse model, which was noted to have decreased expression of both phospholamban and sarcoplasmic reticulum Ca2+-ATPase (SERCA) (12).

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Multiple strategies have been employed to modify altered Ca\(^{2+}\) cycling through calcium-handing proteins as reviewed by del Monte and Hajjar (7). The study of Jagatheesan et al. (8) refers to preliminary data produced in their lab that hypertrophy and cardiac dysfunction are prevented in α-TM Glu180Gly mice crossed with phospholamban-knockout mice and cardiac hypertrophy and hemodynamic performance are improved by gene transfer of SERCA2a into α-TM Glu180Gly neonates. Another group has reported that delayed cardiac myocyte relaxation arising in a different transgenic model of the α-TM Glu180Gly mutation was corrected with the myoplasmic calcium buffer parvalbumin in a genetic cross between mice expressing the α-TM Glu180Gly mutation and mice expressing parvalbumin (4). Calcineurin, a Ca\(^{2+}\)-regulated phosphatase, signals pathological cardiac hypertrophy in response to alterations in Ca\(^{2+}\) handling. Calcineurin inhibition by cyclosporine and FK506 prevented cardiac hypertrophy in transgenic mice overexpressing β-TM, tropomodulin, and myosin light chain-2 in the heart (13). Interestingly, the TM Glu180Gly mice, despite having a severe phenotype, do not have increased levels of activated calcineurin, suggesting a non-calcineurin hypertrophy signaling pathway in this line (12). Further work identifying common and disparate pathways that mediate pathological hypertrophy is likely to provide additional therapeutic targets.

Rather than addressing altered Ca\(^{2+}\) dynamics, Jagatheesan et al. (8) suggest the potential utility of a direct myofilament modification strategy for addressing the pathophysiology of FHC. Is repairing myofilament function by expression of a compensatory myofilament protein a feasible strategy for therapy in humans? Aside from treatment of FHC, other myocardial diseases may respond to repairing defects in myofilament. For example, Day et al. (6) used a designer “histidine button” mutant TnI replacement in transgenic mice to ameliorate the effects of acidosis, ischemia-reperfusion, and post-myocardial infarction cardiac dysfunction. Certainly many barriers exist to translating a myofilament protein replacement strategy for use in humans, the most notable being the difficulty of producing adequate amounts of an abundant structural protein. Hopefully the next decade will permit the identification of additional novel targets in the pathophysiological cascade that produces the devastating phenotype of FHC. The work of Jagatheesan et al. (8) suggests that drugs, peptides, or protein replacement directed at modifying myofilament function may well have a role in these therapies.

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**REFERENCES**