Hypertrophic cardiomyopathy: Translating cellular cross talk into therapeutics

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Hypertrophic cardiomyopathy (HCM) is a common inherited heart disease with serious adverse outcomes, including heart failure, arrhythmias, and sudden cardiac death. The discovery that mutations in sarcomere protein genes cause HCM has enabled the development of mouse models that recapitulate clinical manifestations of disease. Studies in these models have provided unexpected insights into the biophysical and biochemical properties of mutated contractile proteins and may help to improve clinical diagnosis and management of patients with HCM.

Introduction

Discovering the genetic cause for human disease, while often a notable accomplishment, can be akin to a scientific birth announcement—a new name with a future that has more potential than promise. New disease genes and pathogenic mutations often seem far afield from the overt manifestations of human pathology, in particular when the gene is expressed life long, but the disease emerges only after years or decades. The journey from defining genetic etiology to molecular understanding of disease mechanism can be frustratingly slow, requiring exploration of how mutations alter functions of the encoded protein and delineation of the responses that they trigger in relevant cells. Although biochemical and biophysical assays and cell biology experiments advance these insights, full elucidation of the pathophysiology caused by human disease requires a broader approach, including assessment of the impact that cells with a pathogenic mutation have on neighboring cells that do not express the mutant protein and consideration of system-wide responses to a diseased organ. Clinical information from affected patients can inform these responses, albeit with the added complexity of influence from individual genomic variation and life styles. A complementary approach, which we discuss here, is to invest in the development of animal models that are engineered to carry human pathogenic mutations on a single genetic background in a controlled environment.

Sarcomere gene mutations cause hypertrophic cardiomyopathy (HCM)

HCM is a primary genetic disorder of the heart muscle, the leading cause of nonviolent sudden death in young adults, and the most common cause of sudden death in athletes in the US (Maron et al., 1996; Maron, 2004, 2010; Ho, 2010). The prototypic anatomical feature of HCM is left ventricular hypertrophy (LVH), which is usually asymmetric, with greater involvement of the interventricular septum than the left ventricular–free wall (Fig. 1, A and B). LVH is caused by an increase in myocyte size, but not myocyte number, and by a greater amount of myocardial fibrosis, which is distributed throughout the interstium and in discrete foci. HCM myocytes also have distorted nuclei, disorganized myofibrils, and abnormal registration of adjoining myocytes, which combined with increased fibrosis, contributes to a remarkable and characteristic cardiac histopathology (Fig. 1, C and D). These anatomical manifestations adversely impact diastolic function (cardiac relaxation), the quintessential pathophysiologic abnormality observed in HCM that accounts for patient symptoms and contributes to adverse outcomes: arrhythmias that predispose to sudden cardiac death and progression to heart failure.

HCM is caused by a dominant mutation in one of eight genes that encode protein components of the sarcomere (Fig. 1 E; Seidman and Seidman, 2011). Sarcomeres are the fundamental unit of contraction and are composed of thick (myosin) and thin (actin) filaments that slide past each other and produce force (Solaro, 2010). With depolarization of myocytes, calcium enters through the voltage-gated L-type Ca^{2+} channels on the membrane and activates release of stored calcium from the sarcoplasmic reticulum (SR) through the cardiac ryanodine (RyR2), a process termed calcium-induced calcium release. Increased cytosolic calcium binds troponin C, produces conformational changes in the troponin complex, and displaces tropomyosin from the actin–myosin interactive sites. Myosin binds actin and results in formation of cross-bridges, and with hydrolysis of ATP by myosin, energy propels myosin sliding along actin filaments. The resultant force is transmitted to the sarcolemma and extracellular matrix. Upon rebinding of ATP, myosin releases

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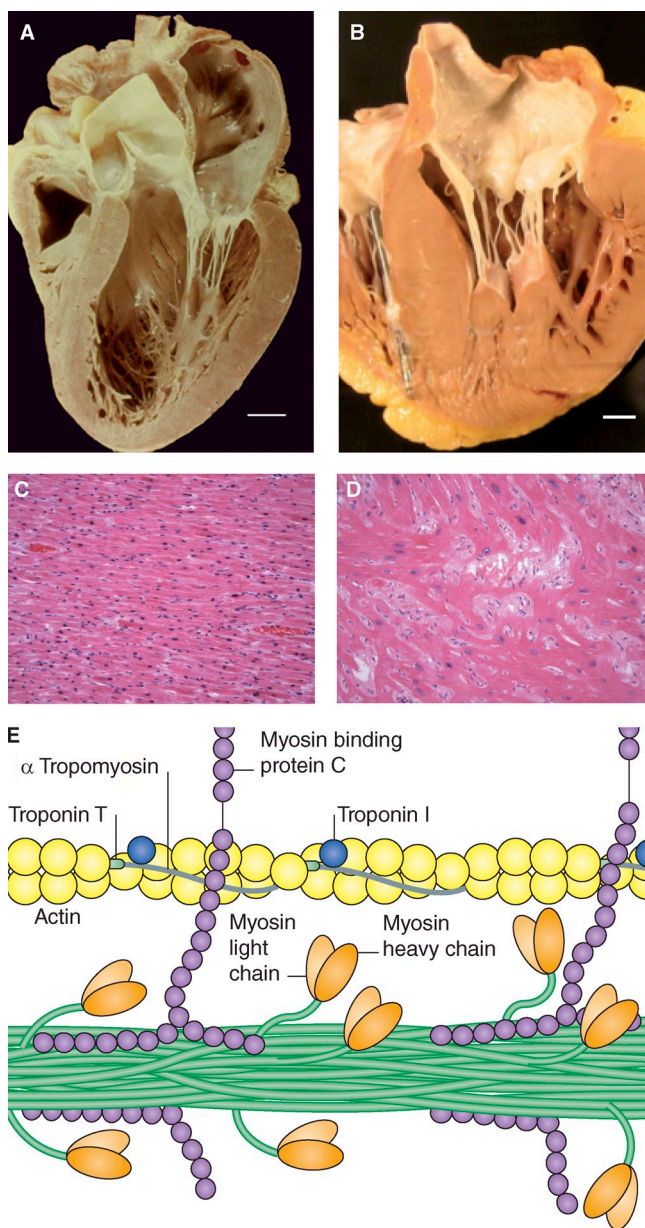www.jcb.org/cgi/doi/10.1083/jcb.201207033

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Figure 1. **Cardiac manifestations and genetic causes of HCM.** (A and B) In comparison to the normal heart (A), the HCM heart (B) shows LVH with increased thickness of the walls and papillary muscles. Bars, 1 cm. The image in A is reprinted from Wang et al. (2010) with permission from *Annals of Internal Medicine*. (C and D) In comparison to normal myocardial histology (C), HCM (D) shows misaligned myocytes with enlarged nuclei and expanded interstitial matrix (light pink). Hematoxylin and eosin stain. (E) A schematic of the sarcomere showing the major contractile proteins that are mutated in HCM.

actin, and calcium recycles back to SR via an ATPase-dependent pump (sarco/endoplasmic reticulum Ca^{2+}-ATPase) or is extruded via the Na^+/Ca^{2+} exchanger, whereas troponins T and I and tropomyosin rebind actin, blocking myosin interactions, collectively resulting in muscle relaxation.

More than 50% of HCM mutations occur in *MHY7*, which encodes β cardiac myosin heavy chain, or *MYBPC3* (Seidman and Seidman, 2011), which encodes myosin binding protein C, a phosphorylatable molecule that regulates sarcomere contraction by affecting the likelihood of cross-bridge binding

(Colson et al., 2010). With the exception of some *MYBPC3* mutations, all HCM mutations encode missense residues that substitute only one amino acid in a sarcomere protein. The discovery of genetic causes for HCM posed a new enigma—how does a subtle change in one protein component of the multimeric sarcomere produce the profound histopathology and symptoms of HCM?

Modeling HCM in mice

The absence of adult ventricular myocyte cell lines has precluded many strategies to delineate the impact of HCM mutations on myocyte structure and function. To overcome this issue, several teams engineered HCM mutations into mice. Our group modeled human β myosin mutations that are associated with severe human phenotypes by introducing missense mutations ($MHC^{Arg403Gln}$, $MHC^{Arg453Cys}$, and $MHC^{Arg719Trp}$; Geisterfer-Lowrance et al., 1996; Debold et al., 2007; Teekakirikul et al., 2010) into the endogenous cardiac α myosin heavy chain gene. Cardiac α and β myosin isoforms are reciprocally expressed during development and across species. In humans, the α isoform predominates during embryogenesis, and the β isoform predominates in postnatal life, whereas in mice, the opposite pattern occurs. Myosin isoforms share 93% amino acid identity, and although their functions in the sarcomere are identical, biophysical and biochemical differences have been described. Despite these issues and differences in cardiac physiology between these species, mice carrying human mutations recapitulate many important aspects of HCM.

Young mutant mice show neither LVH nor histopathology (denoted as prehypertrophic), mirroring the quiescent phase in children with HCM mutations and normal cardiac dimensions. After pubescence, there is insidious emergence of disease, and adult mutant mice have pathological hallmarks of HCM, including LVH, myocyte disarray, and increased amounts of myocardial fibrosis (Geisterfer-Lowrance et al., 1996; Wolf et al., 2005; Teekakirikul et al., 2010).

Primary isolates of myocytes from HCM mice show multiple intrinsic abnormalities. Mutant myocytes have higher end-systolic and end-diastolic stiffness and slower relaxation dynamics compared with myocytes isolated from wild-type littermates (Chuan et al., 2012). Surprisingly, mutant myosins isolated from HCM mice exhibit increased active force generation, increased ATP hydrolysis, and faster actomyosin sliding velocities in comparison to wild-type myosins (Tyska et al., 2000; Debold et al., 2007; Chuan et al., 2012). Together, these data suggest an unexpected conclusion: that HCM mutations increase the biophysical properties of myosin. As such, LVH in HCM does not seem to be a compensatory response to inadequate contractile force production but instead a direct response to HCM mutations.

HCM mutations' impact on myocyte biology extends beyond the sarcomere. Analysis of the mutant mice reveals that an early consequence of a mutant contractile protein is a perturbation of calcium cycling (Fatkin et al., 2000). Although the mechanism that accounts for this is unclear, there is some evidence suggesting that mutant sarcomeres retain calcium for longer times than normal (Sweeney et al., 1994; Lankford et al., 1995; Spindler et al., 1998; Marston, 2011).

Biochemical and biochemical properties of HCM myocytes also impair relaxation. Myosins extracted from MHC[Arg403Gln] mice have enhanced affinity for actin within tropomyosin–troponin–actin complexes (Palmer et al., 2008), an interaction that is expected to exert drag on actin filaments during relaxation and would impair diastolic relaxation (Chuan et al., 2012). Delayed calcium reuptake into the SR also delays relaxation and might activate calcium-dependent signals that promote changes in myocardial architecture. The transcription factor Mef2 (myocyte enhancer factor-2) is increased in HCM, particularly in myocytes that reside near microscopic foci of necrosis and fibrosis (Konno et al., 2010). Activation of Mef2 occurs via calcium-dependent CaMKII (calcium/calmodulin-dependent protein kinase II) phosphorylation and serves as a molecular marker of myocyte stress (Kim et al., 2008). Localized Mef2 expression predestines premature death of HCM myocytes (Konno et al., 2010) and the subsequent accumulation of myocardial fibrosis.

Transcriptional activation of Tgf-β signaling in mouse HCM

Myocardial fibrosis (increased numbers of nonmyocyte cells and amounts of extracellular matrix proteins) is a pathological hallmark of HCM that correlates with the degree of hypertrophy, impaired ventricular performance, diastolic dysfunction, and increased energy consumption. Myocardial fibrosis contributes to progressive heart failure and may increase risk of arrhythmias and sudden cardiac death (Basso et al., 2000; Choudhury et al., 2002). The molecular events involved in the accrual of myocardial fibrosis in HCM has been enigmatic, especially because nonmyocyte cells (presumptive cardiac fibroblasts) that are involved in fibrosis do not express sarcomere proteins and are therefore not directly impacted by HCM mutations.

To define transcriptional responses to HCM mutations that increase cardiac fibrosis, we used next generation sequencing technologies to compare RNAs expressed in nonmyocyte cells isolated from wild-type and HCM mice (Teekakirikul et al., 2010). Surprisingly, >1,500 RNAs that are enriched in nonmyocyte cells are altered by a sarcomere gene mutation. Functional annotation of transcripts with increased expression showed significant overrepresentation ($P < 0.05$) of gene ontogeny terms related to the extracellular matrix, cell cycle, and cell proliferation, including significantly increased expression ($P < 0.0001$) of transcripts that encode fibroblast proteins. Notably, the expression of RNAs encoding Tgf-β1 and 2, periostin, collagen, and connective tissue growth factor was increased, both in nonmyocyte cells isolated from prehypertrophic MHC[Arg403Gln] mice (Kim et al., 2007) and in mice with overt hypertrophy (Teekakirikul et al., 2010). As these molecules exacerbate extracellular matrix production and promote fibrosis in other pathologies, increased expression of proteins by nonmyocyte cells in HCM hearts would be expected to increase myocardial fibrosis.

To evaluate the role of profibrotic molecules in the emergence of HCM, we initially examined MHC[Arg403Gln] mice bred onto a periostin-null background (Oka et al., 2007). Fibrosis in these compound mutant mice was reduced but not extinguished. Next, we treated HCM mice from the prehypertrophic phase

through to the expected development of overt disease with neutralizing antibodies that target all three Tgf-β isoforms. Blocking Tgf-β signaling significantly diminished nonmyocyte proliferation, reduced expression of downstream targets of Tgf-β activation, and resulted in less hypertrophy and histopathology. Additional experiments have indicated that Tgf-β activates the canonical Smad-dependent pathway in HCM mice (Teekakirikul et al., 2010).

To consider whether this strategy could be adapted for human translational experiments, we assessed a pharmacological approach to diminishing Tgf-β signals in HCM mice. The peptide hormone angiotensin II increases cardiac fibrosis via activating Tgf-β signals (Porter and Turner, 2009) and blockade of the angiotensin II type I receptor by the antagonist losartan, a Food and Drug Administration–approved antihypertensive drug, blocks Tgf-β activation and reduces circulating levels (Matt et al., 2009). Losartan administered to prehypertrophic mice and continued throughout life showed benefits comparable with Tgf-β neutralizing antibodies: treatment extinguished periostin expression and nonmyocyte proliferation and reduced LVH and HCM histopathology that emerged in untreated mutant mice. Early pharmacologic intervention was critical; administration of losartan to mice with established HCM was unable to reverse hypertrophy or fibrosis (Teekakirikul et al., 2010).

These studies raise many important and unanswered questions. What triggers increased Tgf-β activation early in the course of HCM? Does Tgf-β activation account for myocyte hypertrophy? By what mechanisms do HCM myocytes increase Tgf-β activation in nonmyocytes? An enticing theory that addresses some of these questions links the enhanced biophysical properties of HCM sarcomeres (Tyska et al., 2000; Chuan et al., 2012) to the transcriptional activation of Tgf-β (Fig. 2). A previous study demonstrates that ex vivo cultures of myocytes isolated from wild-type mice, when subjected to mechanical stretch, increase Tgf-β expression above levels found in quiescent myocytes (Ruwhof et al., 2000). Similarly, isolated nonmyocyte cells subjected to stretch activate Tgf-β expression (van Wamel et al., 2002). Perhaps the greater biomechanical forces produced by HCM mutant sarcomeres in myocytes and transmitted to nonmyocytes increase Tgf-β expression that activates a profibrotic program in HCM hearts. With the expansion of the extracellular matrix–surrounding myocytes, relaxation would be further impaired, and myocyte metabolic demands and cellular stress would increase, resulting in Mef2 activation and ultimately myocyte death and focal myocardial scarring. Recurrence of this life-long scenario could contribute to the LVH and HCM histopathology and promote progressive symptoms in patients.

Translation of basic insights into clinical studies of HCM

Discovery of the genetic cause for HCM combined with robust high-throughput sequencing platforms has enabled a cost-effective gene-based diagnosis for HCM. Comprehensive sequence analyses of all HCM genes in an affected individual can identify the pathogenic mutation and define mutation carrier status in all at-risk family members. As HCM is a dominant disorder, 50% of first-degree relatives of affected individuals are predicted to carry
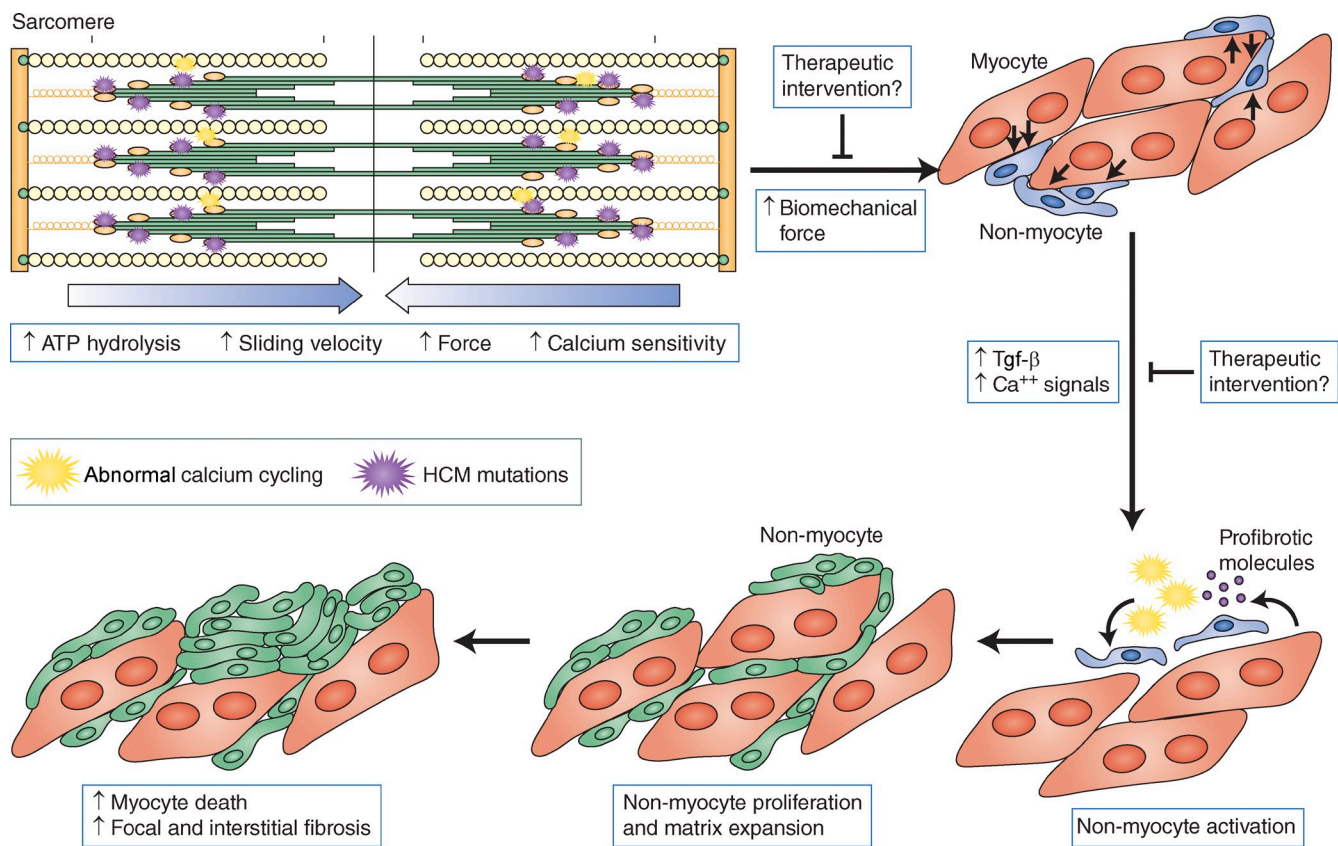
Figure 2. A model by which sarcomere protein mutations cause HCM histopathology. Sarcomere proteins containing HCM mutations (purple) exhibit enhanced biophysical properties of the contractile apparatus and delayed calcium reuptake into the SR. Increased mechanical forces of HCM myocytes, and perhaps calcium-dependent signals, are transmitted to the surrounding nonmyocyte cells and activate transcriptional responses. Transcriptional activation of Tgf-β–dependent signals in nonmyocyte cells promotes proliferation and secretion of profibrotic molecules, which expand the extracellular matrix. Both the intrinsic biophysical properties of mutant sarcomeres and the extrinsic strain imposed by the increased amounts of fibrosis augment myocyte stress (evident by increased Mef2 expression) and promote early myocyte death and subsequent focal scarring. The expanded interstitium and foci of scars perturb myocardial histology and contribute to LVH (Fig. 1, B and D). The interrelated pathophysiology that results from HCM mutations poses several potential therapeutic opportunities. Small molecules that directly target the sarcomere and attenuate the enhanced biophysical properties caused by HCM mutations would be expected to diminish downstream deleterious signals. A less direct approach would be to capitalize on pharmacologic agents that impact myocyte calcium cycling and/or that inhibit Tgf-β activation so as to limit myocardial fibrosis.

the same pathogenic mutation. Clinical evaluations of mutation carriers can reveal occult HCM in asymptomatic adults and/or normal cardiac morphology in young "preclinical" individuals, in whom disease has not yet emerged. Recent detailed imaging experiments and serum biomarkers of preclinical mutation carriers (Ho et al., 2010) recapitulate seminal observations made in experimental models of HCM. Before the onset of hypertrophy, preclinical mutation carriers have increased systolic contraction and diminished relaxation, analogous to increased enhanced biophysical properties of isolated mutant myosins and increased stiffness in myofilament preparations with HCM mutations. Direct assessment of Tgf-β activation has not been undertaken in human HCM, but both preclinical mutation carriers and HCM patients have significantly higher than normal levels of circulating C-terminal propeptide of type I procollagen, a protein associated with increased collagen synthesis and fibrosis (Ho et al., 2010).

Next step—HCM therapeutics

The concordance between human and mouse HCM studies provide strong evidence that fundamental understandings of how HCM alters sarcomere function, myocyte biology, and cardiac performance will provide new therapeutic opportunities. A clinical trial to assess the safety and efficacy of pharmacologic inhibition of Tgf-β signaling in HCM is in development, but additional strategies to address more proximal consequences of sarcomere gene mutations are needed. There are new opportunities to modulate the release of SR calcium, by stabilizing the RyR2 channel and interacting proteins (Shan et al., 2010). Whether these can be exploited to improve calcium cycling in HCM warrants consideration. The most direct approach would be to directly target sarcomere function—a daunting but feasible task, given the recent study of a small molecule that increases myosin activity (Malik et al., 2011). Identification of molecules that attenuate the increased biophysical properties of sarcomere mutations would directly address the primary defect in HCM and potentially abrogate the myriad of downstream signals that underpin HCM pathophysiology. Continued basic studies of sarcomere gene mutations can be expected to uncover other strategies to limit symptoms and improve outcomes in HCM and as well to increase knowledge of cardiac muscle biology.

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