

EDITORIAL COMMENT

Role of the Extracellular Matrix in the Pathogenesis of Hypertrophic Cardiomyopathy*



Ali J. Marian, MD

The prevailing hypothesis in the pathogenesis of human hypertrophic cardiomyopathy (HCM) places the primary abnormality in cardiac myocytes (1). The hypothesis is in accord with the genetic evidence defining HCM mostly as a disease of mutations in genes encoding sarcomere proteins (reviewed in Marian and Braunwald [2]). Sarcomere proteins are predominantly, if not exclusively, expressed in the striated muscles and not other cell types, such as fibroblasts or endothelial cells. Thus, the primary abnormality in HCM has to reside in cardiac myocytes. The pathological and clinical manifestations of HCM, however, are the consequences of intertwined and complex interactions among cellular constituents of the myocardium, including myocytes and fibroblasts. Accordingly, the impetus for the pathogenesis of the histological, functional, and clinical phenotypes originates from cardiac myocytes, the site of expression of mutant sarcomere proteins. The causal mutations by impairing interactions among the protein constituents of

sarcomeres, such as myosin heavy chain (MYH) and actin, alter biochemical and mechanical properties of myofibrils, including calcium sensitivity of myofibrillar adenosine triphosphatase (ATPase) activity, force generation, and relaxation (2). These biochemical and functional perturbations result in activation of stress-sensitive pathways and expression of trophic and mitotic autocrine and paracrine factors that target the cells in the myocardium and induce secondary changes, such as myocyte hypertrophy and interstitial fibrosis, followed by the ensuing clinical phenotypes of cardiac hypertrophy, cardiac arrhythmias, and systolic and diastolic dysfunction (2).

Interstitial fibrosis is a well-recognized and common histological feature of HCM (3). Classically, it is recognized in post-mortem histological examination of HCM hearts and on occasion by measuring circulating cleaved products of collagens (4-6). In recent years, however, cardiac magnetic resonance (CMR) imaging has enabled in vivo assessment of interstitial fibrosis in patients with HCM. The modality enables detecting and quantifying extracellular volume, which is tagged by late gadolinium enhancement (LGE), as a surrogate for myocardial fibrosis. Increased myocardial fibrosis, although secondary to the effects of paracrine factors emanating from cardiac myocytes carrying the pathogenic variants in genes coding for sarcomere proteins, is recognized as a risk factor for cardiac arrhythmias and sudden cardiac death (3,7-13). Myocardial fibrosis is also considered a determinant of diastolic and to a lesser degree systolic dysfunction in HCM and other myocardial diseases (12,13). However, it has not been implicated in the enhancement of myocardial or myocyte contraction, as observed in HCM.

In this issue of *JACC: Basic to Translational Science*, Sewanan et al. (14) provide evidence implicating

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

From the Center for Cardiovascular Genetics, Institute of Molecular Medicine and Department of Medicine, University of Texas Health Sciences Center at Houston, Houston, Texas. Dr. Marian's research programs are supported in part by grants from the National Institutes of Health (NIH), the National Heart, Lung and Blood Institute (NHLBI) R01 HL088498 and 1R01HL132401, the Leducq Foundation (14 CVD 03), The Ewing Halsell Foundation, the George and Mary Josephine Hamman Foundation, and the TexGen Fund from the Greater Houston Community Foundation.

The author attests he is in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* [author instructions page](#).

extracellular matrix (ECM) in the pathogenesis of myocardial mechanical dysfunction in HCM. Using a clever approach, the authors decellularize myocardial strips collected from the hearts of a Yucatan minipig model of HCM, caused by the classic HCM mutation, namely, the p.Arg403Gln, in the gene encoding MYH7 sarcomere protein. They then seed the decellularized myocardial strips with human induced pluripotent stem cell-derived cardiac myocytes (iPSC-CMs) originated from a healthy individual. They culture the seeded cells to generate an engineered heart tissue (EHT) and then characterize effects of ECM originated from HCM hearts on mechanical properties of EHT generated from a normal individual. The authors report that ECM originating from the mini swine hearts with HCM enhanced contraction and impaired relaxation of the healthy EHT. The authors suggest that the ECM from the HCM myocardium stores the memory of the diseased heart and, upon exposure to wild type myocytes, incites phenotypic changes that resemble those of the ECM host (i.e., HCM). The authors conclude that their findings highlight the significant role of the ECM in the pathogenesis of HCM phenotypes.

SEE PAGE 495

The findings of the study by Sewanan et al. (14) are provocative because they indicate that altered ECM in HCM, which is not expected to express the causal mutation, not only impairs relaxation but also enhances contractility of the normal EHT. The extent of these changes is similar to those observed for cardiac myocytes carrying the causal mutation. The findings, therefore, suggest that ECM is a major determinant of the classic functional phenotypes of HCM, including enhanced contraction. Whereas the findings are conceptually in accord with the existence of extensive cross-talks among the heterogeneous group of cells in the myocardium, including cross-talks between myocytes and the ECM, they are observational in nature and require further validation. In addition, they lack mechanistic evidence to support validity of the findings. A notable deficiency is the missing cellular composition of the decellularized tissue prior to seeding of the normal iPSC-CMs. Likewise, there is no information on cellular composition of the EHT prior to functional characterization. Data on cellular composition prior to seeding with iPSC-CMs as well as after extended culture but before functional characterization of EHT would have enabled assessing purity of the preparations in excluding cellular admixture with the residual native myocytes

harboring the *MYH7* mutation and the seeded wild type iPSC-CMs. Equally unclear is the composition of the altered ECM in the HCM samples, besides increased fibrosis. Thus, it remains to be determined which component of ECM is involved and how it affects EHT functions. The authors provide some evidence of altered calcium transient, namely, a 29% increase in time to reach peak calcium transient, which occurs in the absence of significant changes in other characteristics of the calcium transients. Likewise, there were no changes in the transcript levels of selected genes involved in calcium handling. Thus, the mechanism by which ECM from HCM hearts affects function of wild type EHT remains unclear. It also merits noting that the authors did not provide data on the characteristics of the iPSC-CMs and EHT used in these studies. Such data would have been valuable in determining whether these cells and tissues properly represented molecular and functional signatures of adult cardiac myocytes. By and large, myocytes and tissues derived from iPSCs are immature, resembling early fetal cardiac myocytes, and significantly differ from adult cardiac myocytes in epigenetics, gene expression, signal sensing, calcium handling, and excitation-contraction (EC) coupling (15). The latter is particularly pertinent to findings of the present study because iPSC-CMs do not form proper t tubules and sarcoplasmic reticulum, which are necessary for proper calcium handling and EC coupling. Finally, iPSC-CM and EHT lines exhibit significant line-to-line variability and characterization of a single line is insufficient to make firm conclusions. Therefore, as in most if not all initial observations, the intriguing findings of the present study should be considered provisional, pending replication in independent studies and further characterization in multiple lines to reduce and preferably eliminate compounding as well as confounding effects of line-to-line variability, cellular admixture, and effects of decellularization and reseeded processes. Equally important is to delineate the molecular mechanisms by which altered ECM in HCM affects myocyte functions, enhancing its contractile performance while impairing its relaxation. These initial observations could heighten the interest in the role of ECM in the pathogenesis of HCM.

ADDRESS FOR CORRESPONDENCE: Dr. Ali J. Marian, Brown Foundation Institute of Molecular Medicine, The University of Texas Health Sciences Center, 6770 Bertner Street, Suite C900A, Houston, Texas 77030. E-mail: Ali.J.Marian@uth.tmc.edu.

REFERENCES

1. Marian AJ. Pathogenesis of diverse clinical and pathological phenotypes in hypertrophic cardiomyopathy. *Lancet* 2000;355:58-60.
2. Marian AJ, Braunwald E. Hypertrophic cardiomyopathy: genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. *Circ Res* 2017;121:749-70.
3. Briasoulis A, Mallikethi-Reddy S, Palla M, Alesh I, Afonso L. Myocardial fibrosis on cardiac magnetic resonance and cardiac outcomes in hypertrophic cardiomyopathy: a meta-analysis. *Heart* 2015;101:1406-11.
4. Lombardi R, Betocchi S, Losi MA, Tocchetti CG, Aversa M, Miranda M, et al. Myocardial collagen turnover in hypertrophic cardiomyopathy. *Circulation* 2003;108:1455-60.
5. Ho CY, Lopez B, Coelho-Filho OR, Lakdawala NK, Cirino AL, Jarolim P, et al. Myocardial fibrosis as an early manifestation of hypertrophic cardiomyopathy. *N Engl J Med* 2010;363:552-63.
6. Ellims AH, Taylor AJ, Mariani JA, Ling LH, Iles LM, Maeder MT, et al. Evaluating the utility of circulating biomarkers of collagen synthesis in hypertrophic cardiomyopathy. *Circulation. Heart Failure* 2014;7:271-8.
7. Almaas VM, Haugaa KH, Strom EH, Scott H, Dahl CP, Leren TP, et al. Increased amount of interstitial fibrosis predicts ventricular arrhythmias, and is associated with reduced myocardial septal function in patients with obstructive hypertrophic cardiomyopathy. *Europace* 2013;15:1319-27.
8. Chan RH, Maron BJ, Olivetto I, Pencina MJ, Assenza GE, Haas T, et al. Prognostic value of quantitative contrast-enhanced cardiovascular magnetic resonance for the evaluation of sudden death risk in patients with hypertrophic cardiomyopathy. *Circulation* 2014;130:484-95.
9. O'Hanlon R, Grasso A, Roughton M, Moon JC, Clark S, Wage R, et al. Prognostic significance of myocardial fibrosis in hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2010;56:867-74.
10. McLellan AJ, Ellims AH, Prabhu S, Voskoboinik A, Iles LM, Hare JL, et al. Diffuse ventricular fibrosis on cardiac magnetic resonance associates with ventricular tachycardia in patients with hypertrophic cardiomyopathy. *J Cardiovasc Electrophysiol* 2016;27:571-80.
11. Schelbert EB, Piehler KM, Zareba KM, Moon JC, Ugander M, Messroghli DR, et al. Myocardial fibrosis quantified by extracellular volume is associated with subsequent hospitalization for heart failure, death, or both across the spectrum of ejection fraction and heart failure stage. *J Am Heart Assoc* 2015;4(12). pii:e002613.
12. Rommel KP, von Roeder M, Latuscynski K, Oberueck C, Blazek S, Fengler K, et al. Extracellular volume fraction for characterization of patients with heart failure and preserved ejection fraction. *J Am Coll Cardiol* 2016;67:1815-25.
13. Maragiannis D, Alvarez PA, Ghosn MG, Chin K, Hinojosa JJ, Buegler JM, et al. Left ventricular function in patients with hypertrophic cardiomyopathy and its relation to myocardial fibrosis and exercise tolerance. *Int J Cardiovasc Imaging* 2018;34:121-9.
14. Sewanan LR, Schwan J, Kluger J, et al. Extracellular matrix from hypertrophic myocardium provokes impaired twitch dynamics in healthy cardiomyocytes. *J Am Coll Cardiol Basic Trans Science* 2019;4:495-505.
15. Yang X, Pabon L, Murry CE. Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. *Circ Res* 2014;114:511-23.

KEY WORDS engineered heart tissue, fibrosis, hypertrophic cardiomyopathy, induced pluripotent stem cell-cardiomyocytes