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EDITORIAL COMMENT

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

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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 straited 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

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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.

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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 heterogenous 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 reseeding 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.

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