Molecular, Cellular, and Functional Characterization of Myocardial Regions in Hypertrophic Cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is a heterogeneous disease. Although regional functional heterogeneity in HCM is well-known, cellular and molecular regional heterogeneity in human HCM remain unrecognized. We evaluated the molecular and histological heterogeneity as well as mitochondrial function in HCM and their relation to functional heterogeneity in 16 left ventricular segments from a patient with HCM with normal ejection fraction who underwent heart transplantation for advanced heart failure.

Patient
The patient was a 61-year-old man who had a long-standing history of HCM with asymmetrical septal hypertrophy and maximum wall thickness of 26 mm. He underwent myectomy because of severe dyspnea and left ventricular outflow tract dynamic obstruction but was in New York Heart Association functional class IV heart failure despite successful surgery. He received an implantable cardioverter-defibrillator because of risk factors for sudden cardiac death. There was no evidence of epicardial coronary artery disease by angiography. The patient underwent cardiac transplantation because of persistent symptoms, at which time he was on insulin for hyperglycemia and maintenance thyroid replacement. A summary of his hemodynamics and echocardiographic data are shown in online-only Data Supplement Table I. Echocardiography, strain, and strain rate imaging (measured 2–3 hours before the transplantation operation), histopathology, mRNA, and mitochondrial function analyses were assessed. Details of the methodology used appear in the online-only Data Supplement.

Echocardiography, Histopathology, and mRNA Findings
Strain and systolic and diastolic strain rate varied among the different regions of the myocardium. There was a corresponding wide variation with respect to collagen amount, myocyte diameter, and disarray (Figure 1). Segmental variation was noted in the mRNA levels of $COLIA1$ (collagen I) and $COLIIIA$ (collagen III), $TGFB1$ (transforming growth factor-$\beta$), $NPPB$ (brain natriuretic peptide), $MYH7$ ($\beta$-myosin heavy chain [MHC]), $MYH6$ (\(\alpha\)-MHC), and $TTN$ (titin) N2B and N2BA isoforms. The mitochondrial DNA content expressed in relation to cellular DNA, and the mRNA expression of electron transport chain (ETC) enzymes were highly variable (Figure 2). In general, segments with higher collagen content, higher disarray score, and larger myocyte diameter had less compression and expansion (Figure 1). Diastolic strain rate was reduced in segments with higher $\beta$-MHC/$\alpha$-MHC ratio, titin N2B/N2BA ratio, and collagen I and TGF-$\beta$ mRNA levels (Figure 2A and 2B). Likewise, levels of $NDUFB6$ (complex I), $SDHC$ (complex II), and $NDUFS7$ (complex III) mRNA were higher in segments with more diastolic expansion (Figure 2C).

Mitochondrial Function and Its Relation to Myocardial Deformation
For all substrates, the respiratory control ratio was lowest in mitochondria obtained from the septum. In parallel to the gradient seen in mitochondrial respiratory control ratio, deformation was most perturbed in the septum. Online-only Data Supplement Table II shows the state 3/state 4 respiratory control ratio for each substrate.

Discussion
To our knowledge, this is the first report in HCM to show the molecular, metabolic, and cellular basis for the functional heterogeneity in HCM. The findings are consistent with well-known profibrosis effects of TGF-$\beta$ and the adverse effects of collagen I on left ventricular diastolic function. The reduced early diastolic strain rate, which relates inversely to regional stiffness, in segments with a corresponding wide variation with respect to collagen amount, myocyte diameter, and disarray (Figure 1). Segmental variation was noted in the mRNA levels of $COLIA1$ (collagen I) and $COLIIIA$ (collagen III), $TGFB1$ (transforming growth factor-$\beta$), $NPPB$ (brain natriuretic peptide), $MYH7$ ($\beta$-myosin heavy chain [MHC]), $MYH6$ (\(\alpha\)-MHC), and $TTN$ (titin) N2B and N2BA isoforms. The mitochondrial DNA content expressed in relation to cellular DNA, and the mRNA expression of electron transport chain (ETC) enzymes were highly variable (Figure 2). In general, segments with higher collagen content, higher disarray score, and larger myocyte diameter had less compression and expansion (Figure 1). Diastolic strain rate was reduced in segments with higher $\beta$-MHC/$\alpha$-MHC ratio, titin N2B/N2BA ratio, and collagen I and TGF-$\beta$ mRNA levels (Figure 2A and 2B). Likewise, levels of $NDUFB6$ (complex I), $SDHC$ (complex II), and $NDUFS7$ (complex III) mRNA were higher in segments with more diastolic expansion (Figure 2C).
higher titin N2B/N2BA ratio is consistent with the effects of isoform switching on increasing left ventricular stiffness. We noted a lower \(\alpha\)-MHC/\(\beta\)-MHC ratio in segments with worse function, which is consistent with the well-known reduction in \(\alpha\)-MHC levels in cardiac dysfunction.

Assessment of mitochondrial function is a challenging task because it is very difficult to successfully isolate functional mitochondria in humans. The observations provide the most direct assessment of mitochondrial function in HCM. Importantly, the respiratory control ratio for each substrate varied, with the lowest ratio observed in the septum, which indicates that mitochondrial function was most perturbed at that site and was coupled to worse systolic and diastolic function.

Figure 1. A, C, and E, From the midlateral wall, lower extent of disarray, less staining with picrosirius red, and smaller myocytes, respectively, are noted. B, D, and F, In the midseptum, more disarray, more-extensive staining with picrosirius red, and larger myocytes, respectively, are noted. G, A 2D image of the midventricular short-axis view. H, Apical 4-chamber view. I and J, The midseptum shows less radial (yellow asterisk) and longitudinal (yellow arrow) deformation than the midlateral wall radial (green asterisk) and longitudinal (blue arrow) deformation. Echocardiography was performed 2 to 3 hours before the transplantation operation.
Figure 2. A, Segmental mRNA expression of collagen I/III and TGF-β. B, Segmental mRNA expression of α-myosin and β-myosin. C, Segmental mRNA expression of complex I through IV of ETC. ETC indicates electron transport chain; RV, right ventricle; TGF-β, transforming growth factor-β.
Disclosures

None.

References


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