

β -MyHC and Cardiac Hypertrophy Size Does Matter

Kumar Pandya, Oliver Smithies

In response to stress signals, the mammalian heart responds by an increase in its size.¹ This is largely accomplished by an increase in the size of myocytes (hypertrophy) rather than by increasing their numbers (hyperplasia). At the molecular level, pathological stresses induce multiple changes, including genetic reprogramming—the reexpression of a battery of fetal genes and the downregulation of multiple adult genes.² Together, the changes in gene expression result in substantial phenotypic changes, including changes in size, contractility, metabolic state, and electric conductance. Indeed, the reexpression of fetal genes, including β -myosin heavy chain (β -MyHC), atrial natriuretic factor (ANF), and alpha-skeletal actin, has for many years been looked on as an important molecular indicator of pathological hypertrophy.

In spite of this use of β -MyHC reexpression as a marker of pathological hypertrophy, until recently there have been surprisingly few reports on β -MyHC reexpression at the level of individual myocytes within the hypertrophic heart. Nevertheless, previous work using *in situ* hybridization against β -MyHC mRNA has shown that reexpression of β -MyHC does not take place in all areas of the hypertrophic heart; rather, it is distributed in distinct regions.³ More recently, it has been possible to study individual cells from the heart using genetically altered mice in which a fluorescent protein (yellow fluorescent protein [YFP]) is tagged onto the native β -MyHC gene. This procedure confirmed that β -MyHC reexpression takes place in cells located in distinct regions, including regions adjacent to areas of fibrosis and in perivascular areas, although reexpression also occurs to a smaller extent in myocytes seemingly unassociated with fibrosis or vasculature.⁴ Remarkably, these studies showed that cellular hypertrophy is not always accompanied by β -MyHC reexpression. Thus, in the hypertrophic heart, the size distribution of cells expressing β -MyHC was not significantly different from that of cells not expressing β -MyHC, both of which were greater than cells from nonhypertrophic control hearts. This observation was interpreted to mean that signals that are sufficient to induce cellular hypertrophy are not sufficient to induce β -MyHC reexpression, and consequently, that

β -MyHC reexpression is not an obligatory marker of cellular hypertrophy. Nevertheless, the hypertrophic β -MHC-expressing cells were shown to have at least 1 distinguishing characteristic: Their response to β -adrenergic stimulation was less than that of the non- β -MyHC-expressing hypertrophic cells, which had a normal response.⁵

In this issue of *Circulation Research*, a study by Lopez et al⁶ now adds a new and different twist to the relation between β -MyHC reexpression and cellular hypertrophy. Their paper reports detailed analyses of β -MyHC reexpression during pressure overload-induced hypertrophy in mice. To assay β -MyHC expression, the group used an antibody against native β -MyHC that they show faithfully recognizes β -MyHC protein expression in isolated myocytes. The investigators used this antibody for flow cytometric analyses to study β -MyHC reexpression in individual myocytes and for immunohistochemical analyses to study the distribution pattern in tissue sections. The cytometric analyses were made on myocytes prepared by collagenase perfusion, which enabled isolation of large numbers of myocytes from the ventricles of individual hearts (10 000 to 20 000 myocytes/heart). To simultaneously assess cell size, the authors used side-scatter parameters to obtain an unbiased and sensitive sampling of myocyte sizes. This allowed for a very rigorous testing of β -MyHC reexpression in the isolated myocytes coupled with precise estimates of their sizes.

Strikingly and unexpectedly, the group's flow cytometric analyses revealed that β -MyHC reexpression occurs only in nonhypertrophic myocytes, a finding different from previous reports. Thus, as assessed by flow cytometry side scatter, the results showed that the β -MyHC-positive myocytes were smaller in size than the β -MyHC-negative myocytes from the same hypertrophic hearts. Indeed, the β -MyHC-positive myocytes from hypertrophic hearts were the same size as nonhypertrophic myocytes from control animals. This observation differs markedly from previous observations showing that β -MyHC-expressing myocytes were as hypertrophic as non- β -MyHC-expressing myocytes.

The group's immunohistochemical analyses confirmed previous reports that β -myosin heavy chain (MHC) reexpression occurs in subsets of myocytes, distributed around areas of fibrosis and perivascular areas, as well as in myocytes that are unassociated with fibrosis or vessels. An important additional result from their flow cytometric analyses is that the amount of α -MHC in the β -MyHC-expressing cells was the same as in cells not expressing β -MyHC. Thus, the β -MyHC cells have a greater than normal content of MHC.

How might the seemingly contradictory observations regarding cell size be reconciled? The most obvious possibility is that the discordance is related to differences in the models

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and analytic strategies. Lopez et al⁶ suggested that the difference might be explained by sampling, because the studies that used a YFP- β -MyHC model analyzed 600 myocytes, whereas the cytometric study analyzed approximately 250 000 myocytes. However, statistical simulations prompted by this suggestion show that 600 cells with the distribution seen in the YFP- β -MyHC study would have easily detected differences as large as those that Lopez et al report. Thus, differences in cell numbers are not sufficient to explain the differences between the 2 studies. Lopez et al⁶ also suggested that the YFP- β -MyHC reporter used in the earlier study might have created a stress within YFP- β -MyHC-expressing myocytes sufficient to induce hypertrophy. Our experience indicates that this is also unlikely, because YFP- β -MyHC-reexpressing myocytes induced by hypothyroidism are not enlarged. Another possible explanation is that the collagenase treatment used by Lopez et al⁶ to generate a single-cell suspension of myocytes might be less effective in liberating cells from fibrotic areas than from normal nonfibrotic areas. Thus, the collagenase procedure might inadvertently have selected myocytes from less stressed areas of the heart, although the observation that hypertrophic and control hearts yield similar numbers of myocytes argues against this possibility. In contrast, the YFP- β -MyHC studies only analyzed myocytes around fibrotic areas, because β -MyHC reexpression occurred predominantly around areas of fibrosis, rather than in nonfibrotic areas. Finally, the areas of the reexpression detected differ in the 2 studies in the same direction. Thus, more substantial regions of β -MyHC reexpression occurred in nonfibrotic areas in the study by Lopez et al⁶ than in the YFP- β -MyHC study.

Regardless of these uncertainties, the study by Lopez et al⁶ demonstrates that 2 qualitatively distinct myocyte populations exist within the same hypertrophic heart: one that is hypertrophic and predominantly expresses α -MHC and a second that is not hypertrophic and expresses both β - and α -MHC. Taken at its face value, this implies that β -MyHC reexpression is a marker of cellular “normotrophy” under conditions that induce overall cardiac hypertrophy. Possibly the increase in total MHC that results in the β -MyHC myocytes makes them resistant to increases in cell size so that the induction of β -MyHC is in fact protective. Many exciting questions can be imagined moving forward: To what degrees do other members of the fetal gene program follow a similar

discordance vis-à-vis cellular hypertrophy? What are the extracellular determinants, and the attendant molecular pathways, responsible for the induction of this unique nonhypertrophic subset? A rich history of previous work by the same laboratory already points to α -adrenergic receptors as likely suspects.^{7–9} The authors are to be congratulated for adding a new and exciting dimension to the area of genetic reprogramming and cellular hypertrophy.

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None.

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