Phenotyping Hypertrophy
Eschew Obfuscation

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Everyone thinks they know what “cardiac hypertrophy” is: a reactive increase in cardiac size/myocardial mass in response to hemodynamic stress that, in humans, predisposes to early death. Yet, the term “hypertrophy” has become one of the most misused and inaccurate terms in the cardiovascular basic science literature because of its nonspecificity and, as typically used, lack of mechanistic implication. “Hypertrophy” (noun and verb), derived from Greek hyper (above, more than normal) and trophè (nutrition), is defined as “the enlargement or overgrowth of an organ or part due to an increase in size of its constituent cells.” The normal heart is “normal,” and hypertrophy is, by definition, “not normal.” Therefore, normal maturational development at the organ level is not “hypertrophy” (verb) and does not result in cardiac “hypertrophy” (noun), although the cells do “hyper‐trophy” (verb). (Perhaps the term “eutrophy” is more appropriate to describe maturational development.) Likewise, in many genetic in vivo experimental models, the term “cardiac hypertrophy” has too often been loosely applied to any observed cardiac enlargement, frequently with such modifiers as “physiological” or “pathological.” Herein, we reflect on the appropriate meanings of terms and criteria that can be used to more accurately describe cardiac enlargement and myocardial growth, with the anticipation that rigorous mechanistic description of such phenotypes will result in a more coherent appreciation of the parallel and redundant processes that result in “myocardial hypertrophy.”

A Historical Perspective: the Legacy of a Term

The widespread use of the term “hypertrophy” to describe one or a few specific pathophysiological conditions is a holdover from the earliest scientific work on cardiac response to stress, in which cardiac enlargement was largely assumed to be the result of increased cardiomyocyte size and was qualitatively predictable based on the nature of the provocative stimulus. Hence, morphogenic changes in the heart were classified by the nature of the inciting hemodynamic stress as “pressure-overload” or “volume-overload” hypertrophy, referring to concentric hypertrophy (increased thickness of ventricular walls with little or no change in chamber volume) and eccentric hypertrophy (increased chamber volume with ventricular wall thickness increased in proportion with chamber dimensions), respectively. Note that, in both forms of hypertrophy, cardiac dry mass is increased. These etiologic/morphometric descriptors could be further categorized as “compensated” or “decompensated,” based on left ventricular chamber ejection performance and the presumed stage of evolution in an inexorably deteriorating condition. These criteria, while certainly useful in their day, have, in the light of more mechanistic data, become an impediment to understanding and classifying both the genetic and epigenetic processes that lead to cardiomyocyte enlargement.

The relevance of the well-established experimental models of pressure and volume overload to human cardiovascular disease remains undiminished. However, with the advent of directed genetic manipulation of the mammalian genome via gain- and loss-of-function approaches, a reductionist investigative approach for defining the pathways and processes of reactive myocardial adaptation became possible. In summarizing the literature that reports augmentation, inhibition, or ablation of specific candidate “hypertrophy” signaling events through targeted gene deletion or overexpression strategies, one is struck both by the degree of “hypertrophy” obtained purely through signal pathway manipulation and by the varieties of “hypertrophic” phenotypes resulting from perturbation of individual signaling events. In light of the data painstakingly gathered over the last 10 to 12 years, the traditional classifications of hypertrophy can be increasingly irrelevant, and at times, confusing. For example, in some models, there is cellular hyperplasia (increased numbers of normal-sized cardiomyocytes), rather than cellular hypertrophy. Whatever the cellular phenotype, the fundamental characteristic of a grossly “hypertrophied” heart is increased mass, typically measured as whole heart or isolated chamber wet mass. However, an interpretation that increased cardiac mass directly reflects increased cardiomyocyte mass may rely on faulty assumptions, ie, that the water content of the hearts being compared is constant. Although this is likely to be true in the normal in vivo situation (absent local or systemic disease), it may not be true when gross fluid retention occurs, as in florid heart failure. It is also clearly not true when the hearts have been subjected to perfusion with crystalloid, such as in Langendorf or isolated working heart preparations, in which myocardial edema occurs in a predictable, time-dependent manner (G. Dorn, unpublished observation). Experimental rigor dictates that under those circumstances, heart masses should be compared after desiccation. Another fre-
quent assumption is that cardiomyocytes make up the same proportion of myocardium in the samples being compared. This variable may be important in models of myocardial infarction (amylloid, etc), but the most common occurrence is with interstitial or replacement myocardial fibrosis. In either instance, it is possible to determine the presence of abnormal noncardiomyocyte elements in the myocardium by histological examination, hydroxyproline content (to estimate collagen content), or NaOH-insoluble material, and report this as a nonquantifiable variable in the mass measurements.

Increased cardiac size is typically, but not always, accompanied by an increase in cardiac mass. A larger, or “hypertrophied,” heart is usually described as either concentric, eccentric, or dilated, depending on the ratio of left ventricular wall thickness to left ventricular chamber dimension, or h/r.6,13 As noted above, these terms reflect in part the original classification of hypertrophy based on physiological stimulus and also emphasize the importance of chamber geometry in left ventricular pump function via the Laplace relationship.5 However, the terms are purely descriptive and have not helped to define the cellular or molecular characteristics of hypertrophy or to delineate important myotrophic transduction pathways. Indeed, the fundamental determinants of reactive changes in cardiac chamber geometry, ie, ventricular modeling, are even more poorly understood than are the determinants of cardiomyocyte hypertrophy. It is worth mentioning that the most profound cardiomyocyte hypertrophy noted in human heart disease and physiological models has been in dilated cardiomyopathy, not concentric hypertrophy.14,15 Therefore, while it is proper to report the geometrical features of a “hypertrophied” heart because of their functional implications, it may be more correct to assign a separate morphometric classification rather than to use designations of concentric or eccentric as differentiating features of myocardial growth.

Assessment of the functional consequences of reactive cardiac growth is obviously essential to understanding the role of “hypertrophy” on integrated cardiovascular performance and the overall health of the organism. As noted above, the terms compensated and decompensated reflect an assumed stage in the evolution of a disease process and may not be applicable to genetic experimental models, particularly as the bulk of the mechanistic-based (ie, gene manipulation) models lead to disease states relatively quickly, with detectable hypertrophy occurring in a matter of weeks or even days.16,17 Thus, it is reasonable to be cautious in applying the pathways defined by these models to human hypertrophy and functional decompensation. Furthermore, these terms are often used to describe the presence or absence of cardiac failure, not whether cardiomyocyte contractile function is normal or abnormal. Two examples help to explain why these terms may not be terribly useful in mechanistically classifying hypertrophy: Traditional concentric pressure-overload hypertrophy in the “compensated phase” is associated with normal ventricular function but abnormal cardiomyocyte function18 (which may contribute to its ultimate “decompensation”). Second, in the Goq transgenic mouse model, which reproduces many of the cellular and molecular characteristics of pressure-overload hypertrophy, left ventricular pump func-
stances of genetic mouse models where interfering with reactive hypertrophy does not, at least in the short term, compromise ventricular ejection performance after acute pressure overloading.\textsuperscript{21,22} Although the long-term functional consequences of inhibiting reactive hypertrophy in the pressure-overloaded heart still need to be clearly defined, there is currently enough uncertainty that “adaptive” is not a particularly helpful designator. Likewise, “maladaptive” implies that hypertrophy precedes, and therefore leads to, dilated cardiomyopathy. Again, genetically modified mouse models and pacing-induced heart failure in larger animals have shown that this linear temporal relationship is not absolute, and that dilated cardiomyopathies can occur without antecedent hypertrophy.\textsuperscript{12,23}

**Cellular Features**

The hallmark feature of the hypertrophied cardiomyocyte is its increased size, relative to the normal cell. In vitro studies of cultured neonatal cardiomyocytes have shown that the increase in the size of cardiomyocytes subjected to a hypertrophic stimulus is associated with increased sarcomerogenesis and increased expression of natriuretic peptides (both atrial natriuretic peptide [ANP] and B-type natriuretic peptide [BNP]) at both the mRNA and polypeptide levels. Sarcomerogenesis can be assessed immunohistologically. Expression of ANP/BNP mRNA can be measured by transcript assay, ie, Northern analysis, RNase protection analysis, RT-PCR, etc.\textsuperscript{16,17} whereas polypeptide expression can be measured immunohistologically or by radioimmunoassay methods.\textsuperscript{8,9,24} It is not clear how these changes translate to the in vivo adult cardiomyocyte, however. Myofibrillar organization does not predictably change in cardiomyocyte hypertrophy, except in those conditions in which myofibrillar disorganization or disarray occurs. Likewise, although increased ventricular ANP mRNA or protein expression is common in many cardiac disorders, it is not clear that increased ANP is either specific or necessary for cardiomyocyte hypertrophy (see below). However, determining whether an observed increase in myocardial mass results from increased cardiomyocyte size, ie, true hypertrophy, versus an increase in the number of cardiomyocytes, ie, hyperplasia, is critical to understanding the underlying mechanisms for reactive cardiac myotrophy. This is particularly true in the context of recent studies indicating that myocardial or bone marrow stem cells have the capacity to undergo myocardial differentiation\textsuperscript{25} and the likelihood that future therapeutic myocardial regeneration techniques will include targeted application of stem cells to increase myocardial mass.

Cardiomyocyte size can be measured in situ, via fluorescent labeling of the sarcolemma with fluorescein-tagged wheat-germ agglutinin or anti-dystrophin, and computerized assessment of myocyte cross-sectional or long-axis area. Alternately, cardiomyocyte volume can be measured in enzymatically dissociated cells using an automated Coulter counter or as cell capacitance in patch-clamp studies. These methods have the advantage of measuring large numbers of cells per individual heart, which should be considered as replicate determinations from a single sample. However, it is important that in vivo cell geometry is conserved or, less satisfactory, altered in a predictable manner. Somewhat less optimal is manual measurement of length and width of individual cardiomyocytes used for mechanical or calcium cycling studies, although these measurements are preferable to no assessment being made. The relative importance of increased cardiomyocyte cross-sectional area, classically related to “pressure-overload hypertrophy,” compared with increased cardiomyocyte length, most often associated with “volume-overload hypertrophy,” is unknown at this time, although clear indications that sarcomeres are laid down either in parallel or in series are likely important.\textsuperscript{14} In the absence of a detectable increase in cardiomyocyte size, it is not sufficient to conclude that cardiomyocyte hyperplasia exists based simply on increased dry heart weight with normal gross histology. Instead, a diagnosis of hyperplasia should be based on results of a quantitative determination of cardiomyocyte number, typically performed by counting \(\alpha\)-sarcomic actin-staining cells and determining cell number per myocardial area. Nuclear counts may also be helpful in this regard, although most myocardial nuclei are nonmyocyte, and the number of nuclei per cardiomyocyte can vary as a function of age and pathophysiological state.\textsuperscript{26} Strong supporting evidence of cardiomyocyte hyperplasia (or of proliferating/differentiating cardiac stem cells) would be a biochemical marker of active DNA synthesis, such as in vivo nuclear labeling with bromodeoxyuridine (BrDU), although this can also represent DNA repair or an increase in ploidy without cytokinesis.

**Molecular Markers of Cardiac Growth**

It is almost axiomatic that cardiac hypertrophy is associated with, and perhaps in part mediated by, increased expression of a “hypertrophic gene program,” of which ANP, BNP, the \(\beta\)-isoform of myosin heavy chain (\(\beta\)-MHC), and the \(\alpha\) skeletal muscle isoform of actin (\(\alpha\)SA) are prototypical members.\textsuperscript{27} However, the importance of species specificity and developmental stage should be emphasized, particularly for \(\beta\)-MHC. In adult mice and rats, the normal myosin complement is almost exclusively \(\alpha\)-MHC (the “fast” isoform), although \(\beta\)-MHC (the “slow” isoform) is expressed during fetal development. In contrast, in rabbits where both \(\alpha\)-MHC and (predominantly) \(\beta\)-MHC are expressed, thyrotoxicosis induces hypertrophy with a shift toward \(\alpha\)-MHC expression whereas pressure overload induces hypertrophy with a shift toward \(\beta\)-MHC expression.\textsuperscript{28} Note that, in the adult human, \(\beta\)-MHC predominates.\textsuperscript{29} Additionally, most studies of conventionally hypertrophied or failing hearts have shown decreased expression of the calcium cycling protein, SERCA2a.\textsuperscript{30} However, there are now numerous examples of genetically modified mouse models of cardiac hypertrophy in which the classic members of the putative hypertrophy gene program are not expressed as a coherent program. For instance, examples exist of unambiguous cardiac myotrophy (hypertrophy or hyperplasia) with isolated increases in \(\beta\)-MHC and/or \(\alpha\)SA.\textsuperscript{31,32} Likewise, ANP can also be increased independent of or out of proportion to the other members of the presumed “program.”\textsuperscript{33,34} Unfortunately, the variable expression of these genes in different hypertrophy models indicates only that none are necessary for the trophic
process itself, although they are no doubt useful as markers of what transcriptional pathways are being activated in any particular model system. Indeed, ANP or, even more accurately, BNP may be more useful as a highly sensitive marker of cardiac pathology/stress than as a marker of hypertrophy per se. As the global transcriptomic data are accumulated, catalogued, and compared, our understanding of what genes actually mediate what features of cardiomyotrophy will increase. Until then, it remains important to measure ANP, BNP, β-MHC, αSA, and SERCA as a means of comparing seminal transcriptional events in various hypertrophic models. It is not appropriate, however, to measure only one or two of these genes and extrapolate or make generalizations regarding a broader “gene program.” The global interrogation of the transcriptome and proteome holds open the promise of defining the gene/protein subsets that truly reflect the multiple mechanistic underpinnings that we believe exist.

Proposed Criteria for Classifying Cardiac Hypertrophy

We propose that the sine qua non of cardiac/myocardial hypertrophy should be increased cardiomyocyte size, independent of gross heart weight, ventricular morphology, or any particular pattern of gene expression. Any abnormally enlarged heart may be termed “cardiomegalic,” independent of the responsible cellular process, but a determination of the histological/cellular characteristics is necessary before assigning mechanistic labels such as “hypertrophied,” “hypertrophic,” or “infiltrated” (see Figure). Cardiomyocyte size should be quantitatively assessed by histological methods, automated volume determination, and/or whole-cell patch-clamp techniques and be significantly greater than age-matched, same-strain controls. Hyperplasia can be inferred in hearts with increased myocardial dry mass, normal cardiomyocyte size, and no abnormal nonmyocyte myocardial component.

Myocardial hypertrophy can be further classified based on cardiomyocyte contractile function, determined as the rate of unloaded shortening in isolated cells. We recommend that the descriptor pairs “compensated/decompensated” and “physiological/pathological” not be used, in favor of “adaptive/maladaptive.” Our reasoning is that compensation refers to the status of the integrated cardiovascular system of the organism and may change minute by minute based on physiological status/stress, cell autonomous stimuli, or epigenetic processes independent of the primary characteristics of cardiomyocytes. On the other hand, physiological versus pathological is probably the Rosetta stone of hypertrophy investigation, but there is simply not yet enough available information as to what characteristics make hypertrophy one or the other. Fundamental questions that have yet to be answered include whether maturational development and exercise-induced hypertrophy are properly considered together in a single set of “physiological” cardiac responses (we think not). Before these terms can be properly used to describe specific phenotypes, ongoing and future studies must adequately define the relevant molecular and cellular biology. Therefore, we prefer “maladaptive” to describe hypertrophied cardiomyocytes with impaired contractile function and “adaptive” for hypertrophy with normal or even improved function. This is consistent with the known characteristics of “compensated” pressure-overload hypertrophy, in which normal left ventricular ejection performance is associated with depressed isolated cardiomyocyte contractility, and the normal function of cardiomyocytes from athletic hypertrophy.

As noted above, the transcript profile of hypertrophied myocardium will be used eventually to further classify, and ultimately perhaps delineate, specific molecular pathways in individual models. The results of global transcriptome comparisons will no doubt refine our perceptions, but the following rules generally apply to published models: First, there is clearly, as yet, no single gene or set of genes that defines cardiac hypertrophy. The conventional molecular culprits ANP, BNP, αSA, β-MHC, and SERCA have each been observed to be individually or combinatorially regulated in various genetic mouse hypertrophy models. In general, ANP and BNP mRNA tend to be increased in “sicker” hearts, ie, those with depressed ventricular ejection performance or in overt heart failure, with or without hypertrophy (ie, dilated cardiomyopathy). Likewise, increased αSA and decreased SERCA mRNA tend to be observed in hypertrophy models that progress to heart failure. In contrast, β-MHC has been increased in several hypertrophy models with normal cardiac and cardiomyocyte function, indicating that it may not, as conventional wisdom has long held, represent a maladaptive response. Measurements of these cardiac mRNAs should be standard and may help to subclassify hypertrophy phenotypes as suggested above.

However, we are just beginning to recognize the limitations of transcriptome analyses, as well as the identification of the primary genetic etiology; both have provided only limited insight into understanding the resultant “hypertrophic” pathways or pathology. For cardiovascular hypertrophy to be fully understood, we now realize that reductionism has its limitations and the overall intercellular and intracellular regulatory networks in terms of their proteome and component localization will need to be described. Wrapping the profiling data within the biological phenomenology, the so-called “phenome” in which the model under study is completely characterized in terms of the changes at the
cellular, biochemical, organ, and system levels will need to be done to realize the full value of the data. 36 Furthermore, these changes will need to be characterized at as many time points as feasible. As we begin to develop more efficient ways of understanding and perturbing entire networks and the resultant functional consequences, which can culminate in hypertrophy, the reductionist approaches so integral to cardiovascular research in the last half of the twentieth century will become less important.

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References

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