Chips Ahoy
Gene Expression in Failing Hearts Surveyed by High-Density Microarrays

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Organ-level heart failure can result, most obviously, from the death of cardiac myocytes; such death can occur either acutely (with infarction) or more sporadically (in chronic disease). Both types of death can involve a “cell suicide” pathway known as apoptosis. Another key factor for cardiac dysfunction is myocardial fibrosis, which can be, in part, a direct consequence of angiotensin II levels; therefore, a possible benefit of therapy with angiotensin-converting enzyme inhibitors is an improvement in fibrosis. A third generic mechanism for defects in macroscopic or clinically evident cardiac performance arises from changes in the intrinsic mechanical properties of individual cardiac muscle cells. This phenomenon is understood to occur, in part, through changes in the expression of cardiac genes, both subtle and overt, which are collectively referred to as the hypertrophic gene “program.” Reprogramming cardiac gene expression encompasses, among its other features, alterations in (1) contractile proteins of the sarcomere, (2) regulators of calcium handling and other aspects of ion transport, and (3) secreted growth factors and cytokines. Therefore, in pursuit of potential targets for therapy, 2 important aspects of contemporary heart failure research are to discover the signaling pathways that confer adverse responses and to establish a much more comprehensive understanding of the end-organ changes that actually occur.

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In the current issue of Circulation, Yang et al performed a mammoth screen for altered gene expression in heart failure using a newly developed technology, high-density DNA microarrays. Although conceptually simple, these assays are a technical tour de force (Figure). Hundreds, thousands, or tens of thousands of individual DNA segments (presented as short, synthetic DNA strands in their article) are printed on glass slides (“gene chips”) by robotic microfabrication techniques. Messenger RNA is purified (in this case, from normal versus failing hearts), modified with a visualizable tag (in this case, biotinylated nucleotides), and used to label the chips using a fluorescent biotin-binding protein. Levels of expression are therefore monitored simultaneously, as levels of fluorescence overlying each DNA spot, for as many genes as are printed on the slides. Publications have begun to appear that use this technology to monitor gene expression in experimental models of heart disease, including myocardial infarction and hypertrophy. The present study by Yang et al is distinctive in seeking to achieve a similar high-throughput expression profile in human heart failure itself.

For example, with this technique, the expression of mRNA for a gene known to be associated with human heart failure, atrial natriuretic factor, was detected only in the ventricle of failing hearts. More importantly, >5000 different human transcripts were assayed concurrently, and a number of novel discoveries resulted. One gene whose expression was deficient in heart failure was skeletal muscle LIM protein 1 (SLIM1, FHL1). Beyond the microarray studies, which were performed in only 2 normal and 2 failing hearts, the authors substantiated the downregulation of SLIM1 in a larger number of cases using immunoblotting; this technique also ascertained that the protein (and not merely the mRNA that encodes it) is indeed less highly expressed in heart failure.

The LIM motif derives its name from the initials of the 3 transcription factors in which the sequence was first seen (Lin-11, Isl-1, and Mec-3); it comprises 2 cysteine- and histidine-rich zinc-binding regions (“zinc fingers”) and mediates the physical interaction between diverse proteins. Most notably, this mediation includes both interaction with the cytoskeleton and the assembly of multiprotein transcription factor complexes. Some LIM proteins also contain a homedomain that binds DNA directly, a cysteine-rich protein (CRP) domain, a kinase domain for phosphorylation, or additional protein-binding motifs such as a postsynaptic density disc-large zo-1 (PDZ) domain. The PDZ-LIM protein Oracle is most highly expressed in the heart. Interestingly, as shown by the use of null mutations in genetically engineered mice, the LIM-only protein LMO-2 is required for angiogenesis and erythropoiesis, and mice lacking muscle LIM protein (MLP, CRP3) develop dilated cardiomyopathy and skeletal muscle defects. It is still unknown whether this outcome indicates an essential function of MLP in the cytoplasm versus in the nucleus, because MLP is found in both regions of the cell. As was recently reported in this journal, MLP expression is decreased 2- to 3-fold in human dilated or ischemic cardiomyopathy. SLIM1 belongs to the class of striated muscle LIM-only proteins that contain 4.5 LIM domains. Within the cell, SLIM1 is found at focal adhesions and along actin stress fibers. An alternatively spliced human isoform, SLIMMER (SLIM1 with extra regions), contains only 3 LIM domains, adds functional signals for nuclear import and export, and...
High-throughput analysis of gene expression in heart failure by hybridization with high-density DNA arrays.

localizes to the nuclei of undifferentiated myoblasts and the cytoplasm of differentiated myotubes. Most abundant in skeletal muscle, SLIM1 was reportedly localized to the atria in adult rabbit myocardium and, in that species, it was not detected in the ventricle. In the context of that study, the presence of SLIM1 in the human ventricle is unexpected, which may reflect species disparities in chamber-specific expression, differences in the threshold for detection (SLIM1 was found, by in situ hybridization, at low levels in embryonic mouse myocardium) or, conceivably, cross-reactivity with closely related transcripts and proteins. SLIM3 (FHL2, DRAL) is preferentially expressed in the human ventricle; however, the antibody used here was generated against a peptide that is seemingly unique to SLIM1. In contrast with the present report, SLIM1 was reportedly upregulated in mouse models of cardiac hypertrophy (aortic banding) and cardiomyopathy (MLP-deficient mice). In addition, upregulation of SLIM1 has been inferred from the prevalence of transcripts in cDNA libraries of normal versus hypertrophic human hearts. Therefore, additional work to reconcile these differences, including potential clinical disparities in the duration or severity of disease, will be important.

Notwithstanding the remarkable power of profiling gene expression with microarrays, of which the current study is a potent illustration, some limitations are noteworthy, including several discussed by the authors. Low-abundance transcripts that are not detected by more standard hybridization techniques and are found only by reverse transcriptase polymerase chain reaction amplification are unlikely to be seen. Like all other probes and reagents, specificity can be an issue, particularly for gene families with multiple related members, unless the regions incorporated in the array are chosen with this in mind. Beyond these merely technical issues lie 3 broader considerations. First, casting the net for genome-wide changes in gene expression (“transcriptomes”) will inevitably identify variations. Establishing which of these is functionally important will require better informatics (computational approaches to sift through the titanic amounts of raw expression data and help define meaningful patterns) and a more efficient means of confirming gene function experimentally than conventional methods of genetic engineering in mice. Second, changes in mRNA need not accompany changes in the corresponding protein’s abundance or its state of activation. Third, protein microarrays have an additional “blind spot,” at least for the moment. Until all possible genes are both known and represented in arrays, alternative technologies that do not depend on prior knowledge of the genes involved, including subtractive hybridization and serial analysis of gene expression, can bypass this gap in knowledge or reagents and provide a complementary strategy to unmask instructive differences in cardiovascular biology and disease.

References

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