Recounting Cardiac Cellular Composition

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The adult mammalian heart is composed of many cell types, the most abundant being cardiomyocytes, fibroblasts, endothelial cells (ECs), and perivascular cells. Cardiomyocytes occupy ≈70% to 85% of the volume of the mammalian heart.[1–4] Although nonmyocytes occupy a relatively small volume fraction, they are essential for normal heart homeostasis, providing the extracellular matrix, intercellular communication, and vascular supply needed for efficient cardiomyocyte contraction and long-term survival. Both myocytes and nonmyocytes respond to physiological and pathological stress, and maladaptive changes in nonmyocytes, such as cardiac fibrosis or reduced capillary density, participate in the pathogenesis of heart failure.

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Despite the importance of nonmyocytes in cardiac health and disease, there are fundamental gaps in our knowledge about them. For instance, although there has been general consensus that cardiomyocytes constitute 30% to 40% of the cells in the mammalian heart,[5–10] estimates of the frequency of ECs and fibroblasts have differed substantially (Figure). Inadequate tools and lack of reliable markers for fibroblasts have contributed to this uncertainty. In this issue of *Circulation Research*, Pinto et al[9] revisit the question of the cellular composition of the modern heart using modern techniques and reagents. In doing so, they validate a useful new cell surface marker for cardiac fibroblasts, which may prove broadly useful to cardiac biologists.

Several investigators have used stereological methods to estimate the volume fraction of the heart occupied by its major constituent cell types.[1–4,11] In rat or human heart sections imaged by light microscopy, the volume fraction occupied by cardiomyocytes, ECs, and interstitial cells was 70% to 80%, 3.2% to 5.3%, and 1.4% to 1.9%, respectively.[1–3,11] Because the mean volume of cardiomyocytes is 20× to 25× that of ECs or fibroblasts,[1,2] this leads to an estimated proportion of ECs to cardiomyocytes of 0.8 to 1.9 and fibroblasts to cardiomyocytes of 0.4 to 0.7, that is, based on these studies, ECs are among the most abundant cell types in the heart, whereas fibroblasts are 2- to 3-fold less abundant (Figure).

Heart dissociation followed by immunostaining and flow cytometric analysis has also been used to measure the cellular composition of the heart. Using this strategy, Banerjee et al[6] evaluated the composition of the mouse and the rat hearts, marking cardiomyocytes, ECs, and fibroblasts with antibodies to MHCa, CD31, and DDR2, respectively. These investigators concluded that the rat heart comprises 30% cardiomyocytes, 6% ECs, and 64% fibroblasts. Application of the same technique to mouse hearts suggested that ECs were also a minor cell population (6%) in that species as well. Interestingly, the authors found that cardiomyocytes were more abundant in the mouse heart (54%), with correspondingly less fibroblasts (26%). The study suggested that cardiac cellular composition may vary significantly between species, although the proportion of cardiomyocytes measured in the mouse heart is higher than that reported in most other studies.[8–10]

Recently, Bergmann et al[7] analyzed the cellular composition of human hearts by both stereological and flow cytometric methods. The authors marked cardiomyocyte nuclei with PCM1 and ECs with lectin Ulex Europaeus Agglutinin I (UEA), whereas unmarked cells (UEA$^-$ PCM1$^-$) were classified as mesenchymal. Stereological analysis of histological sections estimated the frequency of cardiomyocyte, EC, and mesenchymal populations at 18%, 24%, and 58%, respectively, whereas flow cytometry of isolated cell nuclei estimated frequencies of 33%, 24%, and 43%, respectively. Thus, assuming the most mesenchymal cells are fibroblasts, the Bergmann et al[7] study on human hearts generally corroborated Banerjee et al's[6] report that fibroblasts are the predominant cardiac cell type (presuming that most mesenchymal cells are fibroblasts).

Making sense of these divergent conclusions requires understanding the relative strengths and weaknesses of the techniques used. Flow cytometry is objective, quantitative, and does not have sampling bias. However, the cell dissociation step is critical, and biased recovery of cell types (for example, because of excessive cell death, or incomplete cell dissociation) could strongly influence results. Cell dissociation also destroys morphological and positional information that can assist in identifying cell types. Stereological approaches avoid potential dissociation artifacts, and can incorporate cell position and morphology in cell type identification. However, these histological approaches are often labor intensive, unfamiliar to molecular biologists, and vulnerable to sampling bias. Furthermore, identifying cells solely by morphological information is subjective and prone to error. Both flow cytometry and more recent histological studies rely selective labeling of desired cell populations, either using antibodies or genetic labels. Molecular markers or the antibodies used to detect them may be nonspecific, or alternatively may incompletely label a desired cell type. Thus, the reliability of the reagents and techniques used to classify cells is critical to the interpretation of the experimental data.

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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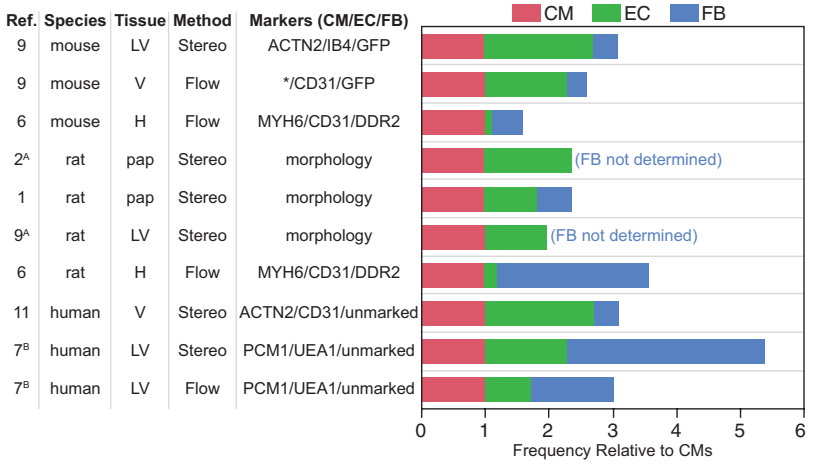

Figure. Cellular composition of the mammalian heart. Summary of studies that have measured the proportion of cardiomyocytes (CMs), endothelial cells (ECs), and fibroblasts (FBs) in the heart, using either stereology or flow cytometry. To allow comparison between studies, results are expressed as relative proportions of cells, with CMs assigned a value of 1. Where numbers of CM nuclei were measured, cell number was not corrected for multinucleation. Additional assumptions used are noted by superscript letters: **A**, CM volume is 25× that of ECs or FBs. **B**, Cells not stained by EC or CM antibodies were FBs. H indicates whole heart; LV, left ventricle; V, ventricles; pap, papillary muscle. *Fraction of CMs not measured and based on stereology study in same reference.

Pinto et al[9] reassessed the cellular composition of the heart by both histology-based and flow cytometric methods. The study incorporated several refinements that make it the most definitive quantification of the cellular composition to date: (1) the study used 3 independent genetically encoded markers of cardiac fibroblasts (PDGFRα^{GFP}, Col1a1-GFP, and Tcf21^{MerCreMer}; Rosa26^{tdTomato}). The PDGFRα^{GFP} and Col1a1-GFP expressing cardiac cell populations overlap to a remarkable degree, suggesting that they each label most cardiac fibroblasts. These reagents are the best current means to identify cardiac fibroblasts.[12,13] In comparison, previously used markers such as DDR2, Thy2, CD90, and Sca-1 are less sensitive and less specific. (2) Cell dissociation was optimized to recover nonmyocytes, rather than to yield viable myocytes. (3) Cells analyzed by flow cytometry were gated viable, nucleated cells, limiting technical artifacts arising from cell debris. (4) Multiparametric flow cytometry data were analyzed using an unbiased clustering algorithm, spanning-tree progression analysis of density-normalized events (SPADE),[14] to identify phenotypically similar groups of cells based on signals from multiple markers.

The study found that 32% of all nuclei in the murine heart belonged to cardiomyocytes (sarcomeric α-actinin), 55% to ECs (nuclei within isolectin B4 outlines), and 13% to fibroblasts (GFP$^+$ in PDGFRα^{GFP} or Col1a1-GFP mice).[9] ECs were similarly abundant in human heart sections. For both species, the abundance of ECs was confirmed using the EC marker DACH1. The abundance of ECs compared with fibroblasts was further independently validated by flow cytometry of the nonmyocyte fraction of murine hearts, followed by SPADE analysis. Of nonmyocytes, 64% were ECs (CD31$^+$ CD45$^-$), 27% were mesenchymal cells (CD31$^-$ CD45$^-$), and 9% were leukocytes (CD45$^+$). The identity of the ECs was further validated by their coexpression of either vascular EC (CD102$^+$ CD105$^+$; 94%) or lymphatic EC (podoplanin$^+$; 5%) markers. About 55% of mesenchymal cells expressed GFP in PDGFRα^{GFP} or Col1a1-GFP mice, supporting their identity as fibroblasts. Thus, the estimates of EC and fibroblast abundance in the heart by histology (55% and 13%, respectively) agreed fairly well to those obtained by flow cytometry (44% and 10%, respectively). Interestingly, these estimates also agree with the relative ratios of cardiomyocytes, ECs, and fibroblasts estimated from initial histological observations made >30 years ago.[1–3,5,11]

Having identified cardiac fibroblasts by flow cytometry, the authors were able to screen for antibodies that could reliably identify them. Neither CD90 nor Sca-1 were sensitive or specific markers, compared with GFP in either PDGFRα^{GFP} or Col1a1-GFP mice. Importantly, the authors found that the antibody MEFSK4 labeled cardiac fibroblasts with high specificity (87%) and sensitivity (97%), when using GFP in PDGFRα^{GFP}/Col1a1-GFP double transgenic mice as the (admittedly imperfect) "gold standard". MEFSK4 is a monoclonal rat antibody developed to immunodeplete murine embryonic feeders from stem cell cultures. This nongenetic cell surface marker of murine cardiac fibroblasts will be useful for studies of cardiac fibrosis. However, the antibody has some limitations. It does not seem to work for immunostaining, and the protein bound by the antibody has not been described. As noted by Pinto et al,[9] subsets of NG2$^+$ mesenchymal cells and CD11b$^+$ leukocytes are also labeled by MEFSK4. The antibody binds to murine embryonic fibroblasts from several strains of mice, so it is likely to label cardiac fibroblasts across mouse strains. However, its usefulness in rats (the host species) or in humans has not been established. Greater experience with MEFSK4 will be needed to determine how well it marks cardiac fibroblasts at different developmental stages or under different pathological contexts.

With the reagents and techniques established by Pinto et al[9] to label and quantify the major nonmyocyte populations of the murine heart in hand, we are better positioned to understand how these cell populations participate in cardiac growth, homeostasis, and disease. During heart development, ECs powerfully influence cardiac morphogenesis and function. The abundance of ECs in the adult heart and their proximity to cardiomyocytes positions them to critically regulate adult heart function and response to pathophysiological stress. Although cardiac fibroblasts may not be as abundant in the normal adult heart as some have previously argued, they nevertheless are important support cells for cardiomyocytes and play central roles in cardiac fibrosis, adverse remodeling, and arrhythmogenesis. Lack of reliable cardiac fibroblast markers has long hampered the study of cardiac fibroblasts, and the work of Pinto et al[9] partially overcomes this hurdle.

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Disclosures

None.

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