Heart failure is the primary cause of adult mortality in the Western world, affecting 1% to 2% of the population, including ≤10% of people >70. Several disease settings, including coronary artery disease, hypertension, diabetes mellitus, and hypertrophic cardiomyopathy, promote a maladaptive response characterized by myocyte hypertrophy and fibrosis. The latter refers to excessive extracellular matrix deposition that causes myocardial stiffening characteristic of heart failure.

Cardiac fibroblasts are the primary cell type responsible for extracellular matrix remodeling during development and in disease. In a pathological context, activation and accumulation of fibroblasts perturb normal deposition and degradation of extracellular matrix components, notably of collagen type I and causes formation of rigid fibrotic lesions within myocardium. Hence, identifying mechanisms responsible for fibroblast accumulation in the failing heart represents a key issue for development of effective antifibrotic therapies.

Recent studies have proposed that endothelial-to-mesenchymal transition (EndoMT) and recruitment of circulating progenitors generate fibroblasts responsible for cardiac fibrosis. A key marker used to identify cardiac fibroblasts in these studies was fibroblast-specific protein 1 that has been recently shown to be expressed by other cell types in the heart, including immune cells. Many of these studies acknowledge that resident fibroblast populations are also engaged in the process of remodeling, but some have implied that these nonresident cell populations are more pathogenic or the “bad guys,” suggesting that these cells contribute significantly to inflammation and matrix production under pathological conditions.

Several markers are currently used to identify cardiac fibroblasts, including discoidin domain receptor family member 2, vimentin, fibroblast-specific protein 1, and Thy1. However, these markers are also expressed by other cell types. Immunostaining against secreted extracellular matrix proteins such as collagen type I and peristin is also commonly used to identify fibroblasts but identification of the producing cell after deposition of the matrix protein is difficult, and vascular smooth muscle cells also produce these proteins to some extent. Recently, more specific cardiac fibroblast markers have been demonstrated, including platelet-derived growth factor receptor α and transcription factor 21. Nonetheless, it seems that the adult cardiac fibroblast population has heterogeneous gene expression, and there may not be any single marker that is uniquely and uniformly expressed. Identifying marker combinations enabling specific labeling of cardiac fibroblasts in healthy and diseased hearts represents a key challenge in the cardiac pathology field.

During development the proepicardium contributes noncardiac mesenchymal progenitors that cover the heart. Some of this mesothelial cell layer, known as the epicardium, undergoes the process of epithelial-to-mesenchymal transition and generates the majority of the ventricular wall cardiac fibroblasts. However, the presence of fibroblasts within myocardium that are not of epicardial origin has not been excluded. Developmental heterogeneity of fibroblasts is observed in valve interstitial cells of the heart, a specialized subset of fibroblasts that constitute the valve leaflets, which have been shown to have multiple embryonic origins including endocardium, epicardium, neural crest, and hematopoietic lineages.

Identifying robust markers as well as establishing embryonic origins of all fibroblasts within various compartments of myocardium is a prerequisite for identifying mechanisms causing fibroblast expansion during fibrosis and ultimately for designing antifibrotic strategies. In the current issue, Ali et al. address these key issues by analyzing origins of Thy1+ (CD90) cardiac fibroblasts in healthy and pressure-overloaded hearts. Thy1, expressed by T-lymphocytes, neurons, and lymphatic endothelium, is expressed in a subset of lung fibroblasts but is thought to be expressed more broadly by cardiac fibroblasts.

Using Cre/JoxP technology to indelibly mark cells expressing Tie2, which predominantly labels endothelial and blood lineages, and selecting for cells positive for Thy1 and negative for endothelial and blood lineage surface markers, Ali et al found a fibroblast population present in adult mouse myocardium at baseline derived from Tie2 lineages. Furthermore, Ali et al report elegant bone marrow transplant and parabiosis experiments, which rule out a significant contribution of hematopoietic and circulating progenitors to fibroblasts after pressure overload. These data are in contrast to previous reports, which implicated a circulating source of fibroblasts identified using a pressure overload model and, more controversially, a myocardial infarction model. From these data, Ali et al conclude that the Tie2Cre-labeled, Thy1+ fibroblasts are derived from endothelium, rather than hematopoietic or circulating blood lineage. Interestingly, this subset represents 20% of fibroblasts at baseline, a percentage that did not vary significantly after pressure overload. From their observations, Ali et al propose that the increased number of endothelially derived fibroblasts observed after aortic banding is primarily because of proliferation of an endogenous fibroblast population rather than...
Whereas Ali et al report that Thy1+, Tie2Cre-labeled fibroblasts signaled led to a decreased number of Tie1Cre lineage traced 1 sig-

HE indicates hematopoietic and endothelial lineage markers. EndoMT or conversion of hematopoietic progenitors into fibroblasts.

After aortic banding, leading to fibrosis. There was no evidence for myocardium. These developmentally derived subsets proliferate and banded animals. Ali et al also show, using Pax3-Cre tagging, that ≈5% of cardiac fibroblasts are derived from neural crest, and that these cells reside mainly in right atrium.

These findings refute the idea that targeting EndoMT in cardiac fibrosis may be beneficial. Previously, Zeisberg et al reported that inhibition of transforming growth factor-β1 signaling led to a decreased number of Tie1Cre lineage traced fibroblast-specific protein 1+ cells in hypertrophic hearts, data that were interpreted as evidence for reduced pathological EndoMT. However, the data presented by Zeisberg et al do not rule out the presence of a Tie1Cre-labeled fibroblast population at baseline, or that the pharmacological treatment attenuated the immune response, and hence the presence of fibroblast-specific protein 1+ immune cells, which would also be labeled by the Tie1Cre marking system. The study of Ali et al supports the main conclusions of another recently reported study. Divergences between the studies include conclusions on the distribution of the resident lineages. Whereas Ali et al report that Thy1+, Tie2Cre-labeled fibroblasts were not preferentially located in any specific area of the adult heart, Moore-Morris et al demonstrated that a subset of fibroblasts migrates from the atrioventricular cushion region into subjacent ventricular myocardium beginning at E12.5 onward, resulting in a complementary distribution of epicardially and endothelially derived fibroblasts, the latter predominating in the adult ventricular septum. This inconsistency may result from the relative difficulty of clearly identifying fibroblasts using Thy1 immunostaining and absence of embryonic tracing to observe the distribution of these cells. Indeed, although Thy1 can be used in flow cytometry and sorting to purify fibroblasts based on exclusion of Thy1+ endothelial and hematopoietic lineages, the strong Thy1 signals detected in vascular and lymphatic endothelium, and resident immune cells make histological examination of fibroblast location using this marker challenging, particularly in adult, remodeling heart. In contrast, the Collagen1a1-GFP reporter used by Moore-Morris et al is absent in endothelial and hematopoietic lineages. In their study, Ali et al reveal that fibroblasts from 2 distinct lineages are present at baseline in adult heart, and that fibrosis in response to pressure overload arises consequent to proliferation of these 2 endogenous populations, rather than resulting from fibroblasts formed from EndoMT, hematopoietic or circulating cells. This changes our view of how cardiac fibrosis develops and emphasizes the importance of targeting activation of resident fibroblast lineages through signaling pathways that are distinct to these cell populations.

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Disclosures
None.

References


Sorting Out Where Fibroblasts Come From
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