Heart failure is the primary cause of adult mortality in the Western world, affecting 1% to 2% of the population, including ≤10% of people >70.4 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.

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 periostin 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/JuxP 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 marbling from circulating Thy1+ fibroblasts.
Whereas Ali et al report that Thy1+, Tie2Cre-labeled fibroblasts
include conclusions on the distribution of the resident lineages.

fibroblast-specific protein 1+ cells in hypertrophic hearts,
βHE indicates hematopoietic and endothelial lineage markers.

HE indicates hematopoietic and endothelial lineage markers.

without evidence for myocardium. These developmentally derived subsets proliferate
after aortic banding, leading to fibrosis. There was no evidence for
EndoMT or conversion of hematopoietic progenitors into fibroblasts.
HE indicates hematopoietic and endothelial lineage markers.

The study by Ali et al shows that Tie2Cre-
tagged Thy1+HE− fibroblasts are present alongside epicardially
derived from epicardium during development, whereas large
numbers of fibroblasts are generated by endothelial-to-
mesenchymal transition (EndoMT) and recruitment of blood cells
after pressure overload. The study by Ali et al shows that Tie2Cre-
tagged Thy1+HE− fibroblasts are present alongside epicardially
derived Tbx18-Cre lineage traced Thy1+HE+ fibroblasts in healthy
myocardium. These developmentally derived subsets proliferate
after aortic banding, leading to fibrosis. There was no evidence for
EndoMT or conversion of hematopoietic progenitors into fibroblasts.
HE indicates hematopoietic and endothelial lineage markers.

These findings refute the idea that targeting EndoMT in
cardiac fibrosis may be beneficial. Previously, Zeisberg et al1
reported that inhibition of transforming growth factor-β1 signal-
ing 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 pathologi-
cal 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.26

The study of Ali et al supports the main conclusions of an-
other recently reported study.27 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 al27 demonstrated that a subset of fibro-
blasts 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 trac-
ing to observe the distribution of these cells. Indeed, although
Thy1 can be used in flow cytometry and sorting to purify fibro-
blasts based on exclusion of Thy1+ endothelial and hematopoietic
lineages, the strong Thy1 signals detected in vascular and
lymphatic endothelium, and resident immune cells make his-
tological examination of fibroblast location using this marker
challenging, particularly in adult, remodeling heart. In contrast,
the Collagen1α1-GFP reporter used by Moore-Morris et al27 is
absent in endothelial and hematopoietic lineages.28

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 prolifer-
ation of these 2 endogenous populations, rather than resulting
from fibroblasts formed from EndoMT, hematopoietic or cir-
culating cells. This changes our view of how cardiac fibrosis
develops and emphasizes the importance of targeting activa-
tion of resident fibroblast lineages through signaling pathways
that are distinct to these cell populations.

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**Disclosures**

None.

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