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Cardiac fibroblasts: from development to heart failure

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Abstract

Cardiac fibroblasts are a major cell population of the heart and are characterized by their capacity to produce extracellular matrix (ECM). In hearts subjected to pressure overload, excessive fibroblast accumulation is responsible for fibrosis of the myocardium, a major clinical issue. Hence, understanding mechanisms generating fibroblasts in this context has become a key question in the cardiovascular field. Recent studies now point to the activation of resident fibroblasts as the underlying cause of fibrosis. However, *de novo* generation of fibroblasts from endothelium and circulating hematopoietic cells has also been proposed to significantly contribute to fibrosis. Here we discuss the latest findings on fibroblast origins, with a particular emphasis on the pressure overload model, and the implication of these findings for the development of anti-fibrotic therapies that are currently lacking.

Heart failure is a major cause of mortality in the western world[1]. Heart failure of diverse etiologies is preceded by adverse remodeling of the heart, involving fibrosis, the excessive deposition of extracellular matrix (ECM) in interstitial and perivascular areas by fibroblasts. In the context of pressure overload, reactive fibrosis causes increased tissue rigidity and ischemia, leading to heart failure.

Although much has been learned about fibroblasts, notably in terms of ECM secretion, electrophysiological properties and signaling (reviewed elsewhere[2,3]), origins of these cells during fibrosis has remained controversial. During development, fibroblasts have been shown to derive from the epicardium[4–8], however, multiple alternative sources of fibroblasts, in addition to resident fibroblasts, have been reported to be involved in pathological remodeling process[9– 11]. Notably, previous reports point to the conversion of endothelium into fibroblasts, known as endothelial to mesenchymal transition (EndoMT), as well as the recruitment of circulating hematopoietic progenitors in failing hearts[9,12,13].

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This review will focus on the current state of the literature on the identification and origins of fibroblasts in hypertensive heart disease, discuss controversies, notably concerning fibroblast markers, and point to future directions.

Cardiac fibroblast: definition and markers

Cardiac fibroblasts perform the essential function of synthesizing the collagen-rich ECM network which provides structural integrity, as well as a source of biomechanical and ECM related signaling[2,14]. Notably, the makeup of the ECM varies during development and into adulthood, promoting myocyte proliferation during early development and hypertrophy after birth.[15] In adult heart, excessive ECM deposition by activated fibroblasts in pathological contexts promotes adverse remodeling of the myocardium[14]. Hence, through their central role in the constitution of the ECM, fibroblasts are key mediators of cell signaling and myocardial remodeling.

Defining the relative proportions of the various cardiac cell lineages has proven challenging, and to date there is no clear consensus. Early studies defining cardiac cell populations, using rat as a model organism, established that the heart is comprised of approximately 70% myocytes and 30% non-myocytes[16]. A subsequent study using flow cytometry analysis of murine heart has shown that 45% of cells are non-myocytes, among which fibroblasts were predominant, and 55% myocytes[17]. However, accurately quantifying relative numbers of non- myocyte cell types, including fibroblasts, endothelial cells, pericytes, smooth muscle cells and resident immune cells, has proven to be more challenging. Indeed, this requires cell-type specific markers, or marker combinations. Although pan lineage-specific markers have long been identified for major cell types such as endothelium (PECAM1,VE-cadherin) and immune cells (CD45), markers for cardiac fibroblasts have remained more controversial.

In addition to “stromal-like” morphological characteristics for identifying fibroblasts, a plethora of molecular markers have been used (Table I). This has resulted from a lack of consensus over how to identify fibroblasts in the absence of more robust markers. Among commonly used markers is Discoidin Receptor 2 (DDR2), a receptor for extracellular matrix proteins, which labels fibroblasts, but not endothelium, smooth muscle or myocytes[18]. However, it is not clear that all fibroblasts are $DDR2^{+}$, and obtaining a specific antibody is challenging. Thymocyte 1 (Thy1, CD90) is also commonly used to identify fibroblasts. Although expressed by cardiac fibroblasts, this receptor is also expressed by immune cells[19], lymphatic endothelium[20] and pericytes[21], making its use limited, at least for immunohistological examination of fibroblasts. The intermediate filament Vimentin has also been used commonly to identify fibroblasts, although it is expressed in many cell types including endothelial cells[22]. The transcription factor TCF21, a marker of proepicardium among other mesothelial populations, has also been used to identify fibroblasts in embryonic heart[23,24,8]. Fibroblast specific protein 1 (FSP1, S100A4) was first identified in fibroblast cell lines[25] and is currently considered a reliable fibroblast marker, but recent publications have shown that, *in vivo*, it labels a subset of immune cells and endothelial cells [26–28].

Using markers of ECM production provides a rational approach to identifying cardiac fibroblasts, as well as their homologues in other tissues, including portal fibroblasts in liver and osteoblasts in bone marrow. ECM is composed of numerous ECM molecules, but mainly consists of heterotrimers of Collagen type I alpha subunits[29]. Recently, a Collagen1a1-GFP reporter[30] has been used to specifically identify cardiac fibroblasts[8,28]. These cells also co-expressed platelet derived growth factor receptor alpha (PDGFRα), which is emerging as a definitive marker of cardiac fibroblasts in adult tissues, and is also strongly expressed in fibroblasts during development and in disease[7,28]. Moreover, PDGFRα is expressed by collagen-producing fibroblasts in skeletal muscle and lung, indicating that it is a robust fibroblast lineage marker[31–33]. Limited expression of this receptor has also been noted in vascular smooth muscle cells in a disease setting[34]. PDGFRα presents major advantages over other markers described above in terms of specificity and expression in fibroblasts regardless of developmental stage and disease state (see Table 1). However, it is important to note that during embryonic development, this receptor is broadly expressed in mesenchymal tissues[35]. The Collagen1a2 inducible Cre driver is another transgenic tool, complementary to the Collagen1a1-GFP reporter, targeting Cre recombinase expression to cells actively expressing collagen[36]. This potentially enables identification and subsequent genetic lineage tracing of fibroblasts with a Cre reporter or conditional gene deletion for functional studies.

Fibroblast markers in the context of fibrosis

Following pressure overload, fibroblasts can adopt an “active” state known as “myofibroblast”. This transition is associated with the expression of a number of markers not expressed by fibroblasts in healthy myocardium. The key marker originally used for identification of myofibroblasts in multiple tissues and pathological contexts is αSMA[37,38,9]. However, this marker is not expressed in all fibroblasts associated with fibrosis, notably following pressure overload[9,28]. The ECM component periostin has also been shown to be secreted by fibroblasts in a pathological context.[39,40] Another marker is fibroblast activation protein-α (FAP), an integral membrane serine protease discovered in the context of epithelial carcinomas[41].

Interestingly, differential expression of fibroblast markers between perivascular and interstitial fibrotic cells of hearts subjected to pressure overload or ischemia/reperfusion (I/R) has been described. TCF21, WT1 and TBX18 are transcription factors expressed in embryonic fibroblast progenitors that are induced in adult cardiac fibrosis. TCF21 is broadly associated with perivascular fibrosis and interstitial fibrosis resulting from pressure overload or ischemic injury. In contrast WT1 and TBX18 are not prevalent in perivascular fibrosis associated with hypertensive disease, but are expressed in interstitial fibroblasts following I/R or aortic banding[42]. Notably, TCF21, WT1 and TBX18 are not expressed in infiltrating immune cells identified by CD45. FSP1 expression is specifically upregulated in perivascular fibroblasts following pressure overload but also can mark infiltrating immune cells and endothelial cells [28]. Finally, stem cell antigen 1 (Sca1) expression has been associated with perivascular (or adventitial) fibroblasts, both in healthy and fibrotic hearts. [43]

Hence, expression of a number of markers is associated with an activated state in fibroblasts. With the exception of TCF21, these markers are not fibroblast-specific. Rather, in combination with fibroblast specific markers including PDGFRα, they provide complementary information on the signaling and properties of specific subsets of fibroblasts within the failing heart.

Developmental origins of cardiac fibroblasts

Epicardium

Until recently, studies focusing on the developmental origin(s) of fibroblasts have been lacking. Several reports showed that cardiac fibroblasts derive from the epicardium, the coelomic mesothelium that covers the heart[4–8]. Epicardium is derived from the proepicardial organ (PEO) that develops in association with the septum transversum at the venous pole of the heart, and is itself a derivative of the splanchnic mesoderm (see Figure 1). It has been shown that the epicardium gives rise to multiple cardiac lineages, including fibroblasts, pericytes, smooth muscle cells and, perhaps, myocytes and endothelium[44,4,6,45,5].

Epicardial cells express a number of markers, including WT1[45], TBX18[6] and TCF21[24]. Heterogeneity in expression of these markers among epicardial cells has been linked to their specification into fibroblast and pericyte/vascular smooth muscle cell lineages. Notably, expression of TCF21 (Epicardin/Pod1/Capsulin) in a subset of epicardial cells is required for specification of epicardially-derived cardiac fibroblasts[8].

Endocardium

The endocardium is a specialized endothelial layer that lines the heart. During development, at least some endocardial progenitors diverge from myocardial lineages at or prior to gastrulation[44,46]. During heart development, subsets of endocardial cells undergo EndoMT to give rise to cushion mesenchyme of atrioventricular canal and outflow tract (see Figure 1) that acts as primitive valves during early development, and will be remodeled to contribute to cardiac valves[47]. Recently, it was shown that fibroblasts generated during this process also invade proximal myocardium, in particular the interventricular septum, a region found to lack substantial numbers of epicardially-derived fibroblasts[28] (see Figure 1). Another study has reported that epicardially-derived valve interstitial fibroblasts are prominent in the parietal (mural) rather than septal leaflets of atrioventricular valves[48]. Hence, mesenchyme associated with the septum, including fibroblasts and valve interstitial cells, includes a large proportion of endocardial cushion derived cells.

Neural Crest

The neural crest is a heterogeneous population of cells that originates from the dorsal aspect of the neural tube. These cells arise from the dorsal aspect of the neural tube and undergo endothelial to mesenchymal transition (EMT), generating various cell types including neurons, glial cells, melanocytes and mesenchymal cells[49]. The cardiac neural crest, a specific subpopulation, plays a key role in morphogenesis of the outflow region of the heart (Figure 1)[50,51]. Genetic lineage tracing in mouse models has confirmed that cardiac

neural crest cells first populate the aorticopulmonary septum and conotruncal cushions before septation and contribute to remodeling, notably by giving rise to valve mesenchyme[52]. Interestingly, this study also demonstrated some neural crest derivatives in mature semilunar valves. Another study has shown persistence of significant number of neural crest derivatives in semilunar and atrioventricular valves, but these cells were melanocytes rather than valve interstitial cells[53]. Recently, a minor subset of neural-crest derived fibroblasts were found within myocardium, mainly residing in right atrium[54].

Origins of fibroblasts during fibrosis associated with pressure overload

Resident fibroblast lineages

Organ fibrosis is a major health issue, entailing multiple studies addressing origins of fibroblasts that accumulate in various pathological contexts. Intense proliferation of resident fibroblasts occurs in the context of pressure overload and heart failure[28,40,54,55]. Lineage studies with several endothelial or epicardial Cres in TAC models demonstrated that developmentally derived endogenous fibroblast populations contribute to fibroblasts responsible for fibrosis. Endothelially derived fibroblasts contributed approximately 15–20% of fibrotic fibroblasts in ventricles, and epicardially derived fibroblasts contributed the remaining 80–85% of the total fibroblast pool in failing hearts, these ratios being comparable to those observed at baseline[28,54]. Consistent with these findings, the two fibroblast populations respond similarly to pressure overload in terms of proliferation and gene expression[28,54]. The foregoing observations demonstrated that fibroblast accumulation results from proliferation of developmentally-derived fibroblast lineages [28,54] (see Figure 2).

Adult endothelial-to-mesenchymal origin

A previous study utilizing FSP1 as a fibroblast marker and a constitutive endothelial/hematopoietic restricted Tie1Cre resulted in the observation of increased numbers of FSP1 positive cells, interpreted to be fibroblasts, that were lineage traced by Tie1Cre, in the setting of pressure overload[9]. These findings were interpreted as evidence that EndoMT from coronary vasculature was a major contributor to cardiac fibroblasts, in this case contributing up to a third of the total fibroblast pool. The recent findings that FSP1 marks substantial numbers of immune cells[26–28], together with the fact that Tie1Cre labels immune cells as well as endothelium[56], suggested that it was important to revisit the occurrence of EndoMT in the setting of pressure overload, utilizing more specific markers of fibroblasts. Toward this end, one study was performed where inducible VE-cadherin CreERT2 was used to lineage label adult endothelium prior to thoracic aortic banding (TAC), with Collagen1a-GFP and PDGFRα as fibroblast markers[28]. Another study relied on Tie2Cre[57] labelling of endothelium in combination with Thy1+CD45−CD31− utilized as a specific signature for cardiac fibroblasts[54]. Results of these studies demonstrated that adult endothelial cells did not contribute to cardiac fibroblasts.

Finally, a recent paper utilizing Col1a2-CreERT2, and DDR2 and Collagen1a1-GFP as markers of cardiac fibroblasts, found that upon cardiac I/R injury, a subset of fibroblasts rapidly up-regulates endothelial markers and undergoes mesenchymal to endothelial

transition (MET)[58], adding a novel twist to the nature of the role played by cardiac fibroblast during fibrosis and their relationship to endothelium. Indeed, this effect is beneficial, and the authors suggest that a subset of fibroblasts act as endothelial progenitors.

Fibroblasts of hematopoietic/circulating origin

In the context of pressure overload, the immune response involves multiple immune cell populations that infiltrate the heart in distinct phases[59]. Since the observation of the presence of circulating "fibrocytes" with mesenchymal-like features such as collagen production[60], several studies have reported a contribution of blood-borne fibroblasts in cardiac fibrosis in various disease models, including pressure overload[13,61,10,62,9]. However, lineage studies with the hematopoietic specific Vav-Cre[28], or with genetically labeled bone marrow transplants[54] and specific markers of fibroblasts demonstrated that circulating hematopoietic cells do not contribute to cardiac fibroblasts in hypertrophic hearts.

Pericytes

Pericytes also represent a potential fibroblast progenitor pool in the context of fibrosis. Pericytes are vascular support cells found associated with smaller vessels that are developmentally close to smooth muscle cells and fibroblasts[4,63] and express markers such as PDGFRβ and NG2[64]. Notably, in other organ systems such as the liver and kidney, pericytes have been shown to give rise to collagen-producing fibroblast[65–67]. Although recent work has suggested this may not the case in heart[68], this questions needs to be directly addressed by lineage tracing.

Conclusion

In conclusion, novel markers for cardiac fibroblasts have enabled recent advances in our understanding of cardiac fibroblast origins during development and in fibrosis. Notably, PDGFRα and the Collagen1a1-GFP reporter appear to be robust cardiac fibroblast markers. Although these seem to label a majority of cells responsible for fibrosis, there appears to be considerable heterogeneity among activated fibroblasts, and future studies may reveal more comprehensive markers, and/or markers specific of a particular disease context.

Lineage tracing using these and other markers has shown that fibroblast heterogeneity is initially acquired during development as a result of two main lineages deriving from epicardium and endothelium. These lineages respond similarly to pressure overload, and proliferate extensively in the regions they initially populate, leading to fibrosis. Interestingly, differential expression of a number of markers, particularly following pressure overload, distinguishes fibroblasts involved in interstitial versus perivascular fibrosis. Furthermore, αSMA clearly labels a subset of "myofibroblasts" following pressure overload, but whether all myofibroblasts are direct descendants of quiescent fibroblasts, or other lineages such as pericytes, remains unclear. Finally, definition of the specific cellular and signaling contributions of immune cell populations should considerably help understand fibrotic processes. Notably, establishing whether any functional overlap exists between

fibroblasts and hematopoietic-derived lineages, currently and area of intense debate, is a key issue.

Much of our current knowledge of fibroblast origins comes from genetic lineage tracing. The use of constitutive Cre drivers targeting progenitor/cell type specific markers provides a means by which to extensively label progenitor populations and their descendants. However, the expression of a number of genes associated with a specific cell type/developmental stage can be more dynamic than expected, in particular in the context of disease, leading to Cre activation and labelling of cells of an uncertain origin [42]. This limitation can be overcome in complementary studies using inducible Cre drivers, in which the timing of Cre expression is under control, but efficiency of excision may be lower.

Thus it appears that targeting resident fibroblast activation/proliferation following pressure overload, rather than EndoMT or circulating fibroblast progenitor recruitment, is a key issue for alleviating fibrosis. This may involve selectively targeting perivascular versus interstitial fibrosis to some extent, as differences in gene expression in fibroblasts in these areas may result from distinct pro-fibrotic signaling. Furthermore, promoting fibroblast clearance in a context where fibrosis is established, for instance by inducing fibroblast apoptosis as occurs in liver fibrosis[69], could provide a means of reversing pathological remodeling in hearts where fibrosis is established.[70]

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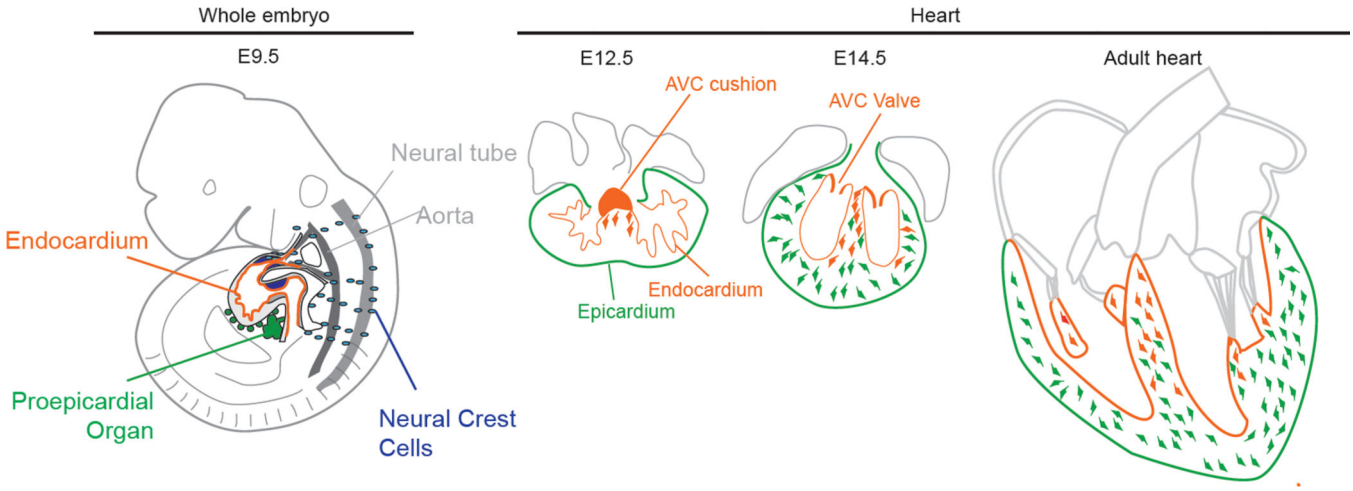

Figure 1.

Lineages giving rise to cardiac fibroblasts during embryonic development. Illustration of an E9.5 embryo showing the myocardium with its inner endocardium, the proepicardial organ and migrating neural crest cells. Fibroblasts derived from the AVC cushion (orange) first invade the septum at E12.5. Subsequently, epicardial EMT generates fibroblasts (green) that invade the free walls by E14.5. In adult heart, endothelially derived fibroblasts are found most abundantly in the septum, whereas epicardially derived fibroblasts populate the free walls.

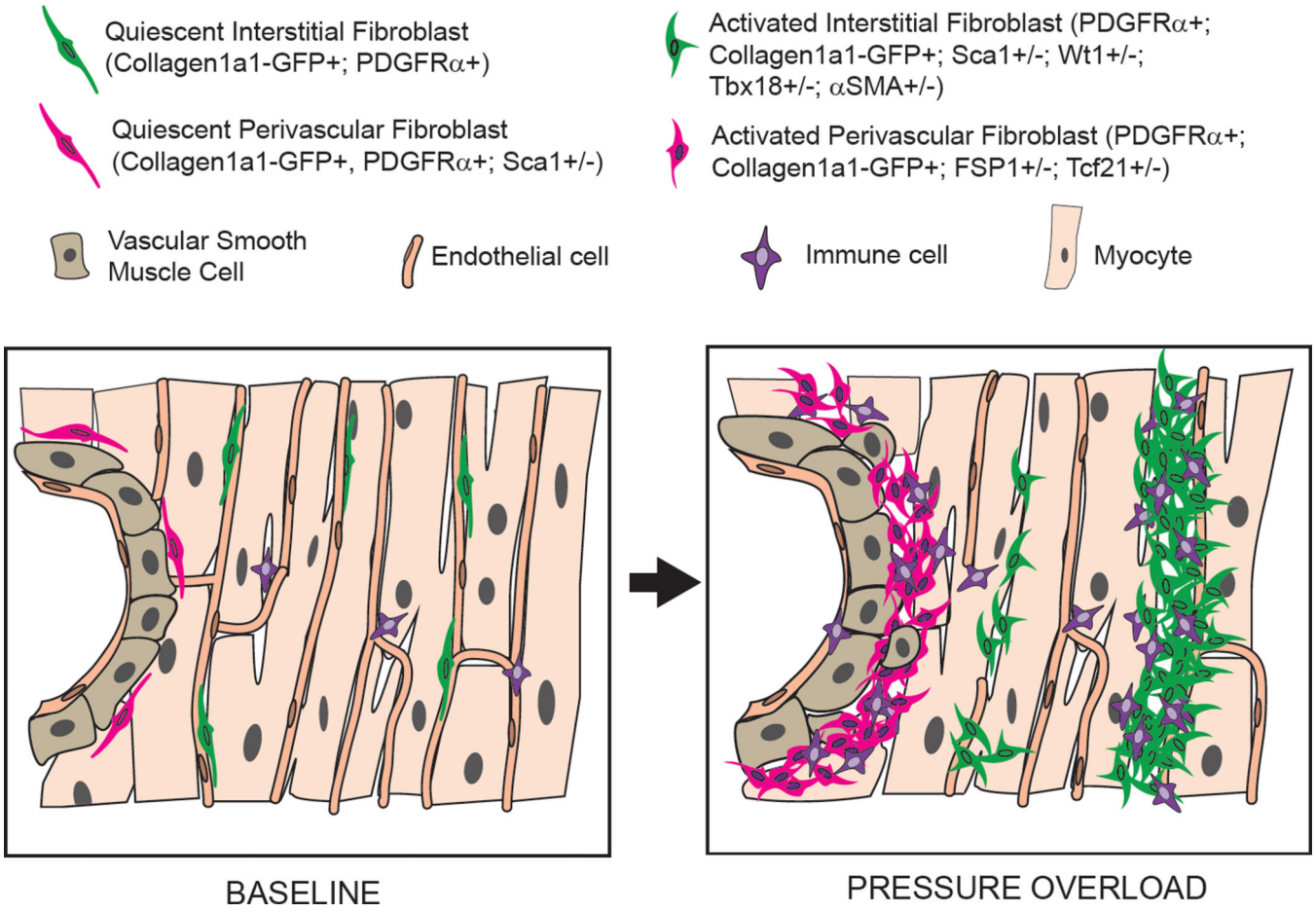

Figure 2.

Interstitial and perivascular fibrosis results from the proliferation of resident fibroblast lineages. Following pressure overload, local signaling from myocytes and non-myocytes, including vascular cells and immune cells, promotes PDGFRα^{+};Collagen1a1-GFP^{+} fibroblast proliferation throughout the myocardium. Several markers have specifically been associated with fibroblasts in interstitial and perivascular fibrotic lesions, but are not expressed by all fibroblasts in these areas (+/−).

Table 1
Fibroblast markers in healthy heart and following pressure overload

The table includes indications regarding the specificity of the markers and references in which they were tested. Markers have been arbitrarily ranked beginning with the overall most robust fibroblast marker, PDGFRα.

	Fibroblasts labelled		Other cell types labelled	
	Healthy adult heart	**Hypertensive disease**	**Healthy adult heart**	**Hypertensive disease**
PDGFRα	All (7,28)	All (7,28)	Epicardium (28)	Epicardium (28)
Collagen 1a1-GFP	All (7,28)	All (7,28)	Epicardium, large vessels (28)	Epicardium, large vessels (28)
Tcf21	All/most (8)	A subset in interstitial and fibrotic lesions	Epicardium (8,42)	Epicardium (42)
DDR2	All (18)	All (18)	Epicardium (71)	Epicardium (72)
Thy1	Most/all (28, 54)	Most/all (28, 54)	Endothelium, immune cells	Endothelium, immune cells
αSMA	No expression	A subset in interstitial lesions (28)	Smooth muscle (38), pericytes	Smooth muscle, pericytes, endothelium (9)
Vimentin	All	All	Multiple cell types including endothelium (22)	Multiple cell types
Wt1	-	A subset in interstitial lesions (42)	Epicardium (42, 73)	Epicardium (42)
Tbx18	-	A subset in interstitial lesions (42)	Epicardium (42)	Epicardium (42)
Periostin (secreted)	-	Associated with areas of interstitial and perivascular fibrosis (39, 74)	Valves (75)	-
FSP1	Rare (9)	A subset in perivascular lesions (28)	Immune cells (28, 27, 9)	Immune cells (28, 27, 9), endothelial cells (9)