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Redefining the identity of cardiac fibroblasts

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Abstract

Cardiac fibroblasts deposit and maintain extracellular matrix during organogenesis and under physiological conditions. In the adult heart, activated cardiac fibroblasts also participate in the healing response after acute myocardial infarction and during chronic disease states characterized by augmented interstitial fibrosis and ventricular remodelling. However, delineation of the characteristics, plasticity, and origins of cardiac fibroblasts is an area of on-going investigation and controversy. A set of genetic mouse models has been developed that specifically addresses the nature of these cells, in terms of both their origins and their response during cardiac disease and ventricular remodelling. As our understanding of cardiac fibroblasts becomes more defined and refined, so does the potential to develop new therapeutic strategies to control fibrosis and adverse ventricular remodelling.

Heart disease is the leading cause of mortality in the developed world. Coronary artery disease and its sequelae, including myocardial infarction (MI) and heart failure, are underlying causes of this lethality[1]. MI injury causes acute necrosis of cardiomyocytes in the affected area of the heart, with concomitant generation of a reparative fibrotic scar that, in the short term, prevents ventricular wall rupture. However, in long-standing heart failure, interstitial fibrosis accumulates throughout the heart, leading to wall and septal stiffening and progressively worsening cardiac function[2]. Acute healing fibrosis after MI and longstanding progressive interstitial fibrosis during heart failure are primarily mediated by the activation and function of fibroblasts[3–5]. The cardiac fibroblast is a plentiful and definable cell type in the heart that expresses a defined array of extracellular matrix (ECM) proteins with type I collagen being the prototypical component. Disease-activated fibroblasts have traditionally been referred to as myofibroblasts, in part because they express contractile genes such as *ACTA2*, which encodes smooth muscle α-actin (α-SMA)[2–5]. These activated fibroblasts also become more highly specialized in the generation and secretion of fibrillar

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collagens and matricellular proteins[2–5]. However, the functional properties of these cells have been largely documented in culture[6]; consequently, the details of how activated fibroblasts perform *in vivo* are still a topic of investigation. Further insights are also needed to determine whether the term myofibroblasts, which designates a contractile cell, is appropriate for fibroblasts in the diseased heart. Indeed, in the injured lung, noncontractile but activated fibroblasts (therefore not myofibroblasts) seem to be even more adept at ECM production and driving the fibrotic response[7].

Possibly owing to a lack of clear definitions for the cardiac fibroblast, many facets of fibroblast biology remain a topic of on-going debates[4,5]. The generation and characterization of genetic mouse models with fibroblast-specific or 'activated fibroblast'-specific allele expression of Cre recombinase has allowed researchers to assess the role of cardiac fibroblasts in the mouse heart in more exact terms. In this Review, we discuss insights gained from the use of genetically engineered mice that allow a systematic evaluation of fibroblast identity[8]. The emerging picture suggests that, despite previous claims that activated fibroblasts transdifferentiate from a wide array of unrelated cell types, the majority of injury-activated, matrix-producing cells seem to expand from tissue-resident fibroblasts that are already present in the myocardium and poised to respond[9–12]. Given that the effectors involved in fibroblast activation have been reviewed previously[13,14], this article focuses primarily on current efforts to define the fibroblast population in the adult myocardium.

Resident cardiac fibroblasts

Initial studies first using morphology and flow cytometry have estimated that fibroblasts constitute between 27% and 50% of the total cells in mouse and rat ventricles, respectively[15–17]. Subsequently, a combination of histological and flow cytometric measurements were used to quantify fibroblasts. Although similar approaches had been used previously, refinements such as optimized tissue digestion, multiparametric analysis, and use of several cardiac fibroblast-specific mouse lines allowed robust calculation of the relative amounts of noncardiomyocytes in the mouse and human hearts. In agreement with some previous findings, endothelial cells far outnumbered the fibroblasts, which were less abundant than previously thought. Approximately 10% of the total cell number in the adult mouse myocardium is comprised of resident adventitial and interstitial fibroblasts[18].

These resident cardiac fibroblasts (FIG. 1) are critical in the structural and mechanical maintenance of the heart. They coordinate the production and remodelling of the collagen network that is critical in ensuring correct conductivity and rhythmicity throughout the heart[2,19,20]. However, the presumed basal functions of fibroblasts in regulating the ECM and structural support of the heart are somewhat implied. Although embryonic loss of fibroblasts results in hearts with substantial reduction in type I and type III collagens, perinatal lethality precludes further investigation into additional roles of fibroblasts in the adult heart[21,22]. Indeed, researchers have largely assumed that activated fibroblasts and/or myofibroblasts (FIG. 1) are the exclusive source of new collagen production (such as type I, II, III, V, and VI collagens). However, this concept needs further mechanistic evaluation *in vivo* because other cell types can make and secrete particular types of collagens, including epithelial and

endothelial cells[23–25], skeletal muscle myotubes and fibers[26–28], and cardiac myocytes[29,30]. Therefore, to characterize all the roles of fibroblasts and activated fibroblasts in the heart, one would need to delete these cells or inactivate them at specific times. Researchers are beginning to undertake these mechanistic approaches in disease models or after acute injury, but little has been done to understand the long-term basal role of tissue-resident fibroblasts in the heart. Moreover, a thorough understanding of the regulation and function of activated fibroblasts or myofibroblasts after acute and chronic injury requires additional investigation (BOX 1).

Another issue in attempting to elucidate the fundamental roles of cardiac fibroblasts is the somewhat nonspecific genetic tools that have previously been used to manipulate the activity or viability of these cells. For example, as discussed below, we have generated mice expressing a tamoxifen-inducible Cre protein from the periostin *(Postn* gene) locus, which was used to induce a diphtheria toxin expression cassette to eliminate newly activated fibroblasts in the heart after MI injury[11]. This approach increased lethality in mice owing to inefficient collagen production and ventricular rupture[11], emphasizing a critical role for activated fibroblasts in wound healing and scar formation.

Previous studies aimed at elucidating the baseline and disease effects of cardiac fibroblasts have been somewhat equivocal, because they relied on markers that were only partially specific to this presumed cell type[8]. For example, discoidin domain-containing receptor 2 (DDR2), fibroblast-specific protein 1 (FSP1, also called protein S100-A4), Thy-1 membrane glycoprotein (CD90), and vimentin, which have all been used in past studies putatively to identify cardiac fibroblasts, are expressed by additional cell types in the heart. FSP1 or its promoter are expressed in many other cardiac cell types, with fibroblasts being in the minority[34]. Thy-1 is expressed on most immune and endothelial cells[35], and DDR2 and vimentin are expressed in endothelial cells[36–38]. Herein lies the difficulty, as researchers had not reached a consensus definition of the fibroblast and its markers. Although still nascent, a consistent group of fibroblast markers is now emerging (FIG. 1) that has led to more consistency in defining this cell type and its function in the heart.

The latest data support the hypothesis that resident fibroblasts derived from the epicardium during embryonic development are the source of disease-relevant activated fibroblasts or myofibroblasts in the adult heart. However, past studies suggested that cardiac fibroblasts originated from heterogeneous pools of cells that required transdifferentiation to achieve a fibroblast phenotype[39]. This latter concept disregards the presence of fibroblasts that already reside in the myocardium to mediate tissue remodelling and ECM production. Moreover, tissue-resident fibroblasts are geometrically interspersed between cardiomyocytes and are of the appropriate molecular programme to allow rapid responsiveness after injury. The hypothesis that activated fibroblasts in the heart emerge from endothelial cells, pericytes, or immune cells would require that they first undergo molecular transdifferentiation, a process that would lack rapid responsiveness and homogenous spatial coverage throughout the tissue.

Embryonic origins of cardiac fibroblasts

Progenitors of resident ventricular fibroblasts invade the mouse myocardium from the epicardium at embryonic (E) day 13.5, with molecularly distinct fibroblasts forming and interspersing throughout the ventricle by E17.5-E18.5[21,22,40–43] (FIG. 2). This embryonic primary source of early epicardial-derived fibroblasts is defined by expression of the marker genes *Tcf21* (encoding transcription factor 21)[22], *Wt1* (Wilms tumor protein)[44,45], and *Tbx18* (T-box transcription factor TBX18)[46]. A second, minor developmental source of fibroblasts from the embryonic endothelium, identified by two groups using lineage tracing, was shown to constitute ~10–20% of the tissue-resident fibroblasts in particular regions of the ventricular septum and right ventricle[10,12] (FIG. 2). Of note, these cells arise early during embryonic development and are not generated by transdifferentiation of mature endothelial cells. In addition to Tcf21, Wt1, and Tbx18 being markers of early embryonic fibroblasts of epicardial origin, platelet-derived growth factor receptor-α (Pdgfr-α)[21] is also used to identify these cells, although only Tcf21 and Pdgfr-α are expressed by resident mature fibroblasts of the adult heart[11,21,22,47].

Origins of activated cardiac fibroblasts

The common method of classifying activated fibroblasts is by detection of gene expression associated with matrix remodelling (encoding selected collagen types, collagen-assembly proteins, and proteases) and/or those expressed by mesenchymal cells (encoding α-SMA, fibronectin, and vimentin). However, the successful detection of these proteins and use to infer fibroblast identity or activation has had technical problems, leading to conclusions that were generally inconsistent between laboratories. The diverse cell types that reportedly give rise to activated fibroblasts include endothelial cells[48,49], bone-marrow-derived fibrocytes or immune cells[50–52], mesenchymal stem/progenitor perivascular cells[53,54], and adult epicardium[55,56] (FIG. 2). Some of these reports examined gene expression of cultured fibroblasts, in which the fibroblast was identified by adhesion to plastic or flow cytometry. Others have relied predominantly on immunohistochemistry with some form of lineage tracing. Surprisingly, few studies implicated the resident fibroblast as a contributor to cardiac remodelling despite their fairly high abundance and obvious capacity to differentiate quickly into myofibroblasts without the need for transdifferentiation[16,18].

Recent studies have demonstrated that the past conclusions regarding extracardiac sources and transdifferentiation of endothelial cells as major contributors to fibrotic activities might not be quantitatively accurate[10–12] (FIG. 2). Multiple research groups, using bone-marrow chimeras, parabiosis, and bone-marrow-specific Cre lines, have reported either no or only modest contribution of bone-marrow-derived cells to the injury-responsive fibroblast population[10–12]. For example, lineage tracing of bone-marrow-derived cells with LysM-Cre[57] or Kit-Cre[58] showed no significant contribution of these lineages to the periostin-expressing activated fibroblasts after MI injury[11]. Similar results were obtained using Vav-Cre lineage tracing that demonstrated little contribution of the haematopoietic lineages to activated fibroblasts in the hearts of mice subjected to pressure-overload stress[10]. Further experiments with bone-marrow chimerism and parabiosis also showed minimal contribution

of bone-marrow-derived cells to the proliferating fibroblast population after pressure overload[12].

Endothelial-to-mesenchymal transition has been proposed as a mechanism for fibroblast generation after MI and experimental diabetes mellitus on the basis of endothelial lineage tracing with Tie1-Cre and Tie2-Cre lines, but both of these studies also relied on FSP1 to identify fibroblasts (FIG. 2)[10,34]. FSP1 is expressed in multiple cell types and is not a specific indicator of the fibroblast lineage *in vivo*, and the Tie1-Cre and Tie2-Cre lines are also expressed in immune cell lineages[10,34]. With the use of a range of distinct endothelial Cre lines, several reports now show that endothelial cell conversion to fibroblasts after injury is very low. When Cdh5-Cre, VE-cadherin-CreERT2 or Tie2-Cre lineage tagging was used to label pre-existing vascular endothelial cells, no fibroblasts were found to arise from this population after pressure overload or MI injury[10–12]. Finally, using single-cell RNAseq, endothelial, smooth muscle, and haematopoietic lineage cells were found not to have a marker profile consistent with an activated fibroblast (defined by a-SMA, periostin, and vimentin expression)[11].

Interestingly, Ubil and colleagues reported that fibroblasts in the mouse heart, as lineage traced using a Col1a2-CreERT2 transgene, generated new endothelial cells in the infarcted myocardium, indicating mesenchymal-to-endothelial transition[59]. However, Kanisicak and colleagues did not observe re-expression of the endothelial cell marker CD31 after MI in two distinct cardiac fibroblast lineages, suggesting that tissue-resident and activated fibroblasts do not generate CD31-expressing endothelial cells[11]. After MI, smooth muscle cells and fibroblasts emerged from adult epicardium[56,60], but after pressure overload by thoracic aortic constriction, few fibroblasts were found to originate from adult epicardium[10,12]. Data generated by ndependent laboratories using lineage tracing and chimeric mouse models, therefore, suggests hat injury-activated, matrix-producing cells from sources such as endothelium, smooth muscle, bone marrow, and blood might be substantially less abundant than previously thought (FIG. 2).

Injury-induced fibroblasts in the heart

More recent studies have made a convincing case that tissue-resident fibroblasts are the primary source of activated fibroblasts underlying tissue fibrosis and disease remodelling in the heart[10–12,60]. First, Kanisicak and colleagues used a newly generated genetic tool in which a tamoxifen-regulated mutated oestrogen receptor-Cre (mCrem) was inserted into the *Postn* locus. This process generated a highly specific means of lineage tracing essentially all activated fibroblasts in the heart subjected to pressure overload, MI injury, or neuroendocrine stimulation, without labelling of other cell types[11]. Using the *Postn*^{mCrem} allele together with a ROSA26^{DTA}, cellular killing allele[61], activated fibroblasts were shown to be required for healing and scar formation after MI injury *in vivo*[11]. The same study also showed that the tissue-resident fibroblasts already in the heart were the overwhelming source of activated fibroblasts after MI injury, pressure overload, or infusion of a fibrosis-promoting neuroendocrine agonist cocktail[11] (FIG. 2). Of note, during development and in other tissues, periostin is not exclusive to activated fibroblasts[31–33]. However, in adult, stressed cardiac ventricles, periostin is uniquely expressed by activated fibroblasts[11].

The *Tcf21* genetic locus was also used to express a tamoxifen-regulated Cre protein for lineage tracing, but this time to show that the overwhelming majority of tissue-resident fibroblasts in the heart were of developmental epicardial origin, and that this Cre-expressing knock-in allele was highly specific in the heart[11,22] (FIG. 3). In support of this conclusion, *Wt1*$^{Cre\text{-}EGFP}$ lineage-traced epicardial derivatives[44] also generated the vast majority of tissue-resident fibroblasts in the heart after pressure overload[10]. Although *Wt1* and *Tbx18* are expressed in embryonic fibroblast progenitors, these genes are not expressed by most mature fibroblasts in the adult heart[62,63]. However, *Tcf21* continues to be expressed by adult cardiac fibroblasts[47] (FIG. 3).

When investigating the endothelial origin of the ventricular septal and right ventricular fibroblasts, two groups have demonstrated that these fibroblast populations can produce ECM in response to pressure overload[10,12]. Compared with the epicardial-derived fibroblasts, gene expression and proliferation were similar in the two cell populations after injury. In conclusion, the tissue-resident fibroblasts generated during development are the primary source of the majority of activated fibroblasts after cardiac injury. Activated tissue-resident fibroblasts also secrete factors that affect cardiac hypertrophy, but that are also likely to enhance the acute injury response and scar formation (reviewed previously[14,64]).

Human cardiac fibroblasts and fibrosis

Much of our knowledge about cardiac fibroblasts has been derived from studies using experimental animal models. Indeed, we know considerably less about the biology of human cardiac fibroblasts. Many studies on human cardiac fibroblasts have relied on primary *in vitro* culture techniques for their expansion and subsequent manipulation[6]. Other studies have examined fibroblasts using histological analysis of post-mortem hearts and the use of α-SMA to identify myofibroblasts[65]. One study using morphometric analysis demonstrated that mesenchymal cells, which include fibroblasts and pericytes, comprise roughly 50% of the cells in the healthy human heart. This study used ^{14}C dating of cells from pathology-free, post-mortem hearts to calculate fibroblast longevity. The entire population of mesenchymal cells was estimated to be renewed about twice during a normal human lifespan and that numbers of these cells peak at age 30 years and then decline[66].

Although post-mortem studies can provide useful information about fibroblast location and numbers, lack of definitive methods to identify cardiac fibroblasts in the human heart is still problematic. The alternative is to measure the products of fibroblast activity by either imaging or biochemical assays. The noninvasive visualization of fibrotic scarring in the heart using MRI has become widespread, and has suggested that the pattern of scarring is dependent on the severity and type of insult[67]. A second method for monitoring emergent fibrosis is measurement of circulating factors[68]. Indeed, some biomarkers have been identified that can be used to indicate the extent of matrix deposition, although how this parameter might correlate with total fibroblast content in the heart is uncertain[14].

Given that ECM production or remodelling is a hallmark of fibroblast activation, a number of individual ECM, remodelling, and matricellular proteins have been proposed as markers for fibrosis, although some studies have not identified a reliable correlation between the

amount of myocardial fibrosis and circulating collagen components[69–71]. A combination of biomarkers predicting type I collagen crosslinking (ratio of circulating type I collagen to matrix metalloproteinase 1 [MMP1; also known as interstitial collagenase]) and deposition (procollagen type I peptide) has been examined. In this study, poor outcomes were associated with low type I collagen crosslinking and high type I collagen deposition in patients with hypertension and heart failure[72]. Another complication is that some presumed fibrosis-indicating proteins can be secreted by inflammatory cells or possibly even other cell types present in the myocardium during injury or longstanding fibrotic disease. Galectin 3, a proposed mediator of vascular fibrosis and inflammation, might be a more reliable biomarker for heart failure with associated fibrosis[73,74]. The fibrosis-associated microRNAs miR-21 and miR-19b have been suggested as circulating measures of cardiac fibrosis and heart failure[75,76]. However, the current measures of fibroblast number and activity in the human heart, as well as total fibrotic content, remain unreliable and often indeterminate. Further molecular dissection of cardiac fibroblasts in animal models is likely to suggest alternative markers for application to human hearts to improve evaluation and tracking of ongoing disease, which is important for advances in treatment of fibrotic disease states with emerging therapeutic agents or strategies.

Unanswered questions

Using the newly generated and verified genetic lineage-tracing mouse models discussed throughout this Review, researchers can now more reliably address fundamental concepts in fibroblast biology that remain unexplored. One very simple question is what happens to activated fibroblasts in a heart with injury resolution after MI, such as those cells within a stable scar after the acute healing process? The same question holds for the fate of activated fibroblasts in hearts when a pressure-overload stimulus is removed. The two possibilities are cellular apoptosis or reversion of the cell to its previous basal state. Studies have shown a dramatic expansion of activated fibroblast numbers in the heart from tissue-resident fibroblasts after MI injury or pressure-overload[10,11]. Kanisicak and colleagues also conducted a specific experiment in which fibroblasts were activated and lineage traced in hearts with the *Postn*^{mCrem} mouse line during angiotensin II and phenylephrine infusion, followed by histological analysis 2 weeks after the infusion was stopped so that the injury response regressed. The data showed that lineage-traced fibroblasts from the activation period were still present in the heart, and that they had a molecular programme more similar to a resident fibroblast than an activated fibroblast[11]. However, total *Postn*^{mCrem} lineage-traced fibroblast numbers were also substantially reduced, suggestive of cellular loss[11]. Many activated fibroblasts are, therefore, likely to undergo apoptosis after injury resolution *in vivo*, but some revert back to a baseline, ‘resting’ state. These concepts need additional in-depth analysis to understand the dynamics of fibroblast differentiation plasticity *in vivo*. Related to this concept, it is intriguing why fully differentiated, a-SMA-expressing fibroblasts lack cellular stability and longevity, suggesting that they are not a truly differentiated cell type as classically defined, but are instead a ‘supraphysiological’ state that requires continuous input for their maintenance. However, myofibroblasts resident in the scar of an MI-injured heart might also remain indefinitely, given the ongoing stress and strain environment of the scar. If the contractile activity of myofibroblasts is indeed

important, their presence might help to maintain scar tension and stability over time (BOX 1).

Another question is whether stable, intermediate states of fibroblast activation exist, or instead whether a scalable continuum of differentiation states exist that interpret the degree of cytokine and mechanical stimulation. For example, one of the first events in fibroblast activation seems to be periostin expression, followed by α-SMA expression[11]. One possible means of examining the question of lineage substates is single-cell RNA sequencing of activated fibroblasts isolated from the hearts of *Postn*^{mCrem} lineage-traced mice[11]. These cells could be removed for analysis from the same region at different time points after injury, or from different regions of the heart so that cells in the scar that experience greater mechanical load could be compared with cells in the border zone with increased compliance. These gene-expression profiles could be clustered in an attempt to identify stable, intermediate differentiated states based on marker genes, or to build a model of a more fluid continuum of differentiated gene expression.

As discussed previously, fibroblasts are likely to be the primary mediators of the production of selected ECM proteins in the heart, as well as in most other tissues in vertebrate organisms. However, cardiomyocytes contain a basal lamina of selected ECM proteins (especially fibronectin, but also α1 type I collagen and type VI collagen) that form early in cardiac development before fibroblasts enter the heart. Moreover, lower organisms such *Drosophila* lack the fibroblast cell type despite having full capacity to mount a fibrotic response with collagen secretion by tissue parenchymal cells[77–81]. Therefore, whether fibroblasts are the only relevant source of collagen and ECM production in the heart, either at baseline or with acute and chronic injury, remains to be tested. Indeed, mammalian cardiac myocytes can express and secrete collagens that could easily contribute to healing in the border areas of an MI[29,30].

The capacity of activated fibroblasts to contract and remodel the heart *in vivo* through expression of smooth muscle-related contractile genes is also of unproven mechanistic importance. This unanswered question has direct implications in naming activated fibroblasts as myofibroblasts or possibly something else. Indeed, mitogen-activated protein kinase-activated protein kinase 2 (*Mapkapk2*)-deficient mice lack expression of important contractile genes in their activated fibroblasts during lung injury, even though these activated fibroblasts produce ECM even more abundantly than control fibroblasts replete in *Mapkapk2*[7]. In the future, it will be interesting to distinguish the ECM-producing functions of activated fibroblasts from their presumed contractile activity in the heart.

Conclusions

Many cellular functions have been attributed to cardiac fibroblasts during homeostasis and cardiovascular remodelling, but the lack of a clear definition of a fibroblast might have led to inaccurate assumptions about this population. Our improved capacity to track fibroblasts *in vivo* is now providing exciting new avenues to understand and target maladaptive fibroblast activities in the heart, as well as potentially important adaptive or physiological functions. A stronger grasp of the identity and behaviour of cardiac fibroblasts and their different

functional stages with defined molecular signatures will be critical in generating and evaluating new antifibrotic therapies for cardiovascular disease in humans, as well as designing new biomarkers to track fibrotic remodelling and evaluate treatment efficacy.

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Author biographies

Jeffery D. Molkentin, PhD. Is a Professor in the Department of pediatrics, University of Cincinnati and Cincinnati Children's Hospital (USA). He received his PhD from the Medical College of Wisconsin in 1994, after which he performed postdoctoral training with Dr. Eric Olson in Texas from 1994–1997, followed by his first faculty appointment in 1997 at the Cincinnati Children's Hospital Medical Center where he remains today. Dr. Molkentin is a full investigator of the Howard Hughes Medical Institute since 2008. Dr Molkentin has placed more than 20 of his past trainees into academics as laboratory principle investigators. Dr. Molkentin's research program continues to focus on the identification of candidate genes and signaling pathways involved in cardiac hypertrophy, contractility, cell death, heart failure, fibrosis and muscular dystrophy, as well as mitochondria-dependent necrosis.

Michelle D. Tallquist Ph.D is an Associate Professor in the Department of Medicine, in the Center for Cardiovascular Research at the University of Hawaii. She received a BA in Chemistry from Kalamazoo College in 1989. After obtaining her degree, she worked several years as an analytical chemist at The Upjohn Company. In 1992 she entered graduate school at the Mayo Clinic, where she studied immunology. For her thesis work she investigated the molecular basis of graft rejection. Her post-doctoral work was carried out in Seattle at the Fred Hutchinson Cancer Research Center where she used the power of mouse molecular genetics to investigate Platelet Derived Growth Factor signaling in vivo. For 10 years, she was faculty at the University of Texas Southwestern Medical Center in Dallas. Her research focuses on understanding the signals that direct fibroblast differentiation and activation during organogenesis and disease processes. Using the mouse, she has defined signaling pathways that are necessary for cardiac fibroblast formation and is generating models to manipulate fibroblast numbers and activation during tissue homeostasis and disease. The long term goal of her research is to understand the beneficial and detrimental actions of the extracellular matrix and growth factors produced by fibroblasts and to identify signaling pathways unique to the fibroblast

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Box 1 | How are fibroblasts activated?

In response to cardiac injury, both cytokine and neurohumoral factors are released and profound changes occur in the mechanical strain relationships within the ventricular wall (or septum), which together are thought to underlie fibroblast activation. A central cytokine involved in fibroblast activation, at least as defined in cultured fibroblasts, is transforming growth factor-β (TGF-β)[82]. TGF-β binds to and causes heterodimerization of TGF-β receptor 1 and 2 that in turn causes direct phosphorylation of the cytoplasmically-localized transcription factors SMAD2/3, which then translocate to the nucleus in complex with SMAD4 to promote fibroblast differentiation-specific gene expression[83]. TGF-β receptor signalling as well as signalling from other neurohumoral factor receptors, such as angiotensin II receptor, also initiate fibroblast activation through engagement of mitogen-activated protein kinase (MAPK) effectors, such p38 (REFS 82,83). For example, activation of p38 in fibroblasts and other mesenchymal cell types induces collagen and smooth muscle a-actin (a-SMA) transcriptional activity as well as the appearance of α-SMA stress fibres[84–86]. A priority is to elucidate the entire molecular circuitry that regulates fibroblast activation, with the aim of identifying novel therapeutic approaches that might limit progressive cardiac fibrosis, as well as different stages of fibroblast differentiation in longstanding disease.

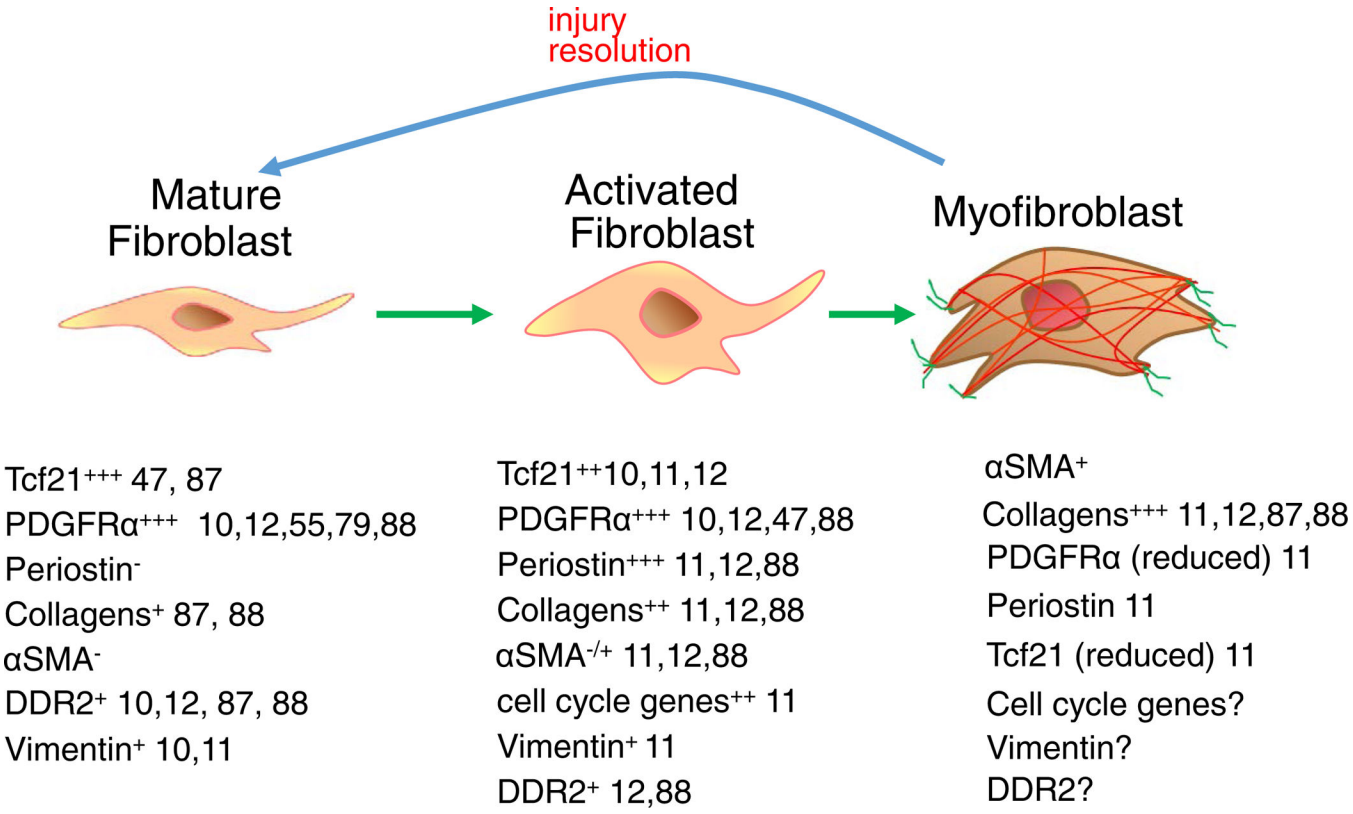

Figure 1 |. Current categories of resident cardiac fibroblasts.

At least three gene-expression profiles can be used to describe fibroblasts in the adult heart[10–12,87,88]. Mature, resident fibroblasts are interspersed in the myomesial space throughout the heart and are proposed to maintain the extracellular matrix (ECM). They have a low level of proliferation and do not express smooth muscle α-actin (α-SMA) or periostin. After injury, a population of fibroblasts, often associated with inflammatory cell accumulation and cardiomyocyte death, rapidly proliferate and become activated to express many of the gene products shown. Later in the response, a smaller population of these activated fibroblasts further differentiate into presumed myofibroblasts, which express α-SMA and other genetic signatures shown. In the past, these myofibroblasts were reported to have increased ECM deposition and contractile capacities[89], although *in vivo* contractile activity has been predominantly documented in skin myofibroblasts[90]. Myofibroblasts have also been shown to regress their activated gene-expression profile and morphology back to the more 'quiescent' and resident fibroblast state[11]. Ddr2, discoidin domain-containing receptor 2; NA, not available; Pdgfr-α, platelet-derived growth factor receptor-α; Tcf21; transcription factor 21.

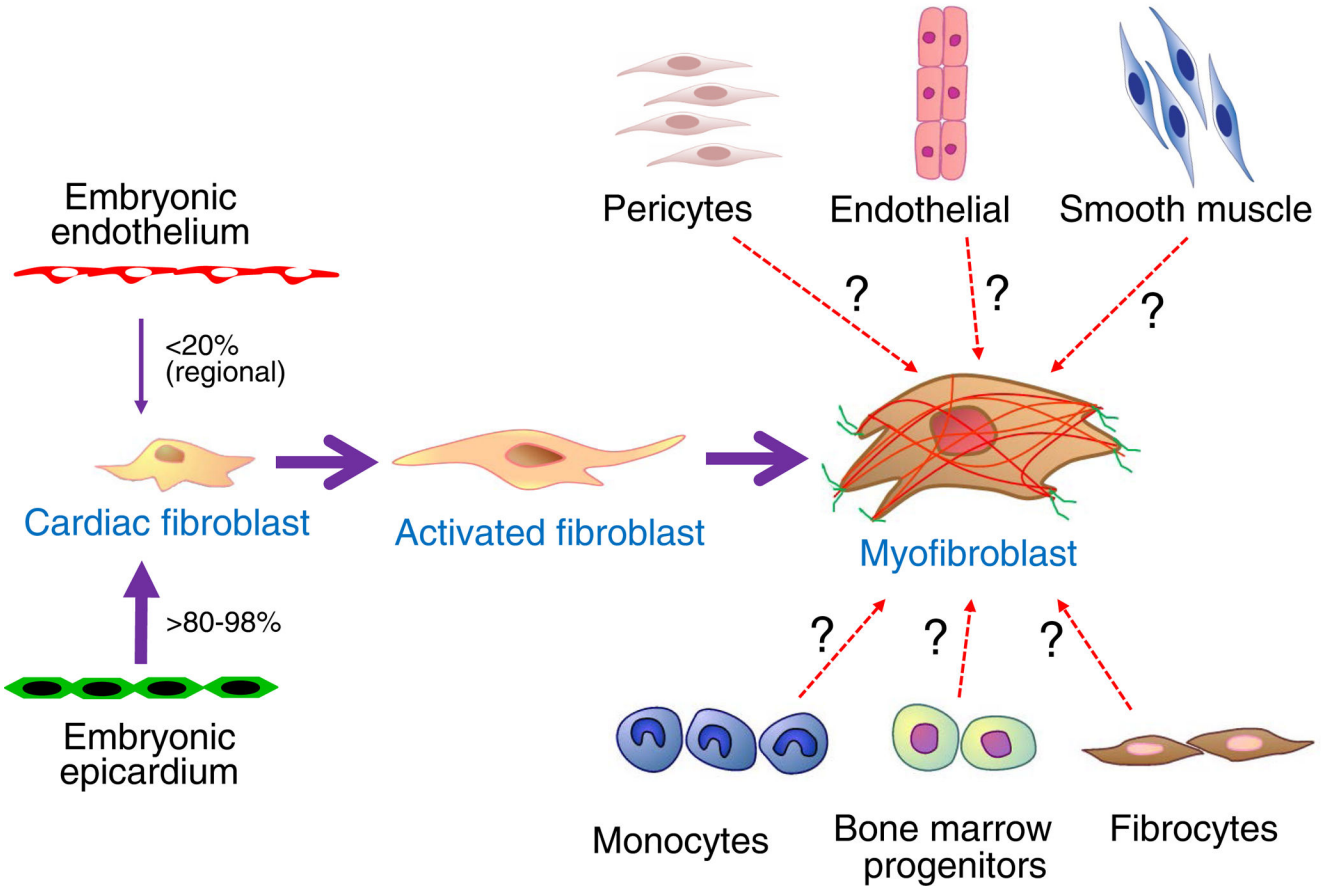

Figure 2 |. Developmental and alternative sources of fibroblasts.

There are two documented sources of tissue resident cardiac fibroblasts, the developmental epicardium and developmental endothelial cells. The epicardial-derived fibroblasts emerge by the process of epithelial-to-mesenchymal transition of the epicardium at embryonic day E13.5 in the mouse, and constitute a majority of the ventricular and atrial cardiac fibroblasts. How the endothelial-derived cardiac fibroblasts arise during development is uncertain, but they contribute tissue-resident fibroblasts to regions of the right ventricle and of the ventricular septum. Studies have suggested a number of nonfibroblast cellular sources, such as endothelial cells, smooth muscle cells, monocytes, fibrocytes, and bone-marrow progenitors (designated with question marks), as the primary origin for newly generated activated fibroblasts and myofibroblasts in the heart after injury. However, recent studies with more highly refined genetic markers have not confirmed these results, and instead have shown that tissue-resident cardiac fibroblasts of developmental origin generate all the activated fibroblasts and myofibroblasts after injury. Pericytes have been reported to have fibroblast-like qualities in the heart when activated, but further work is needed to understand the potential role of these cells[54].

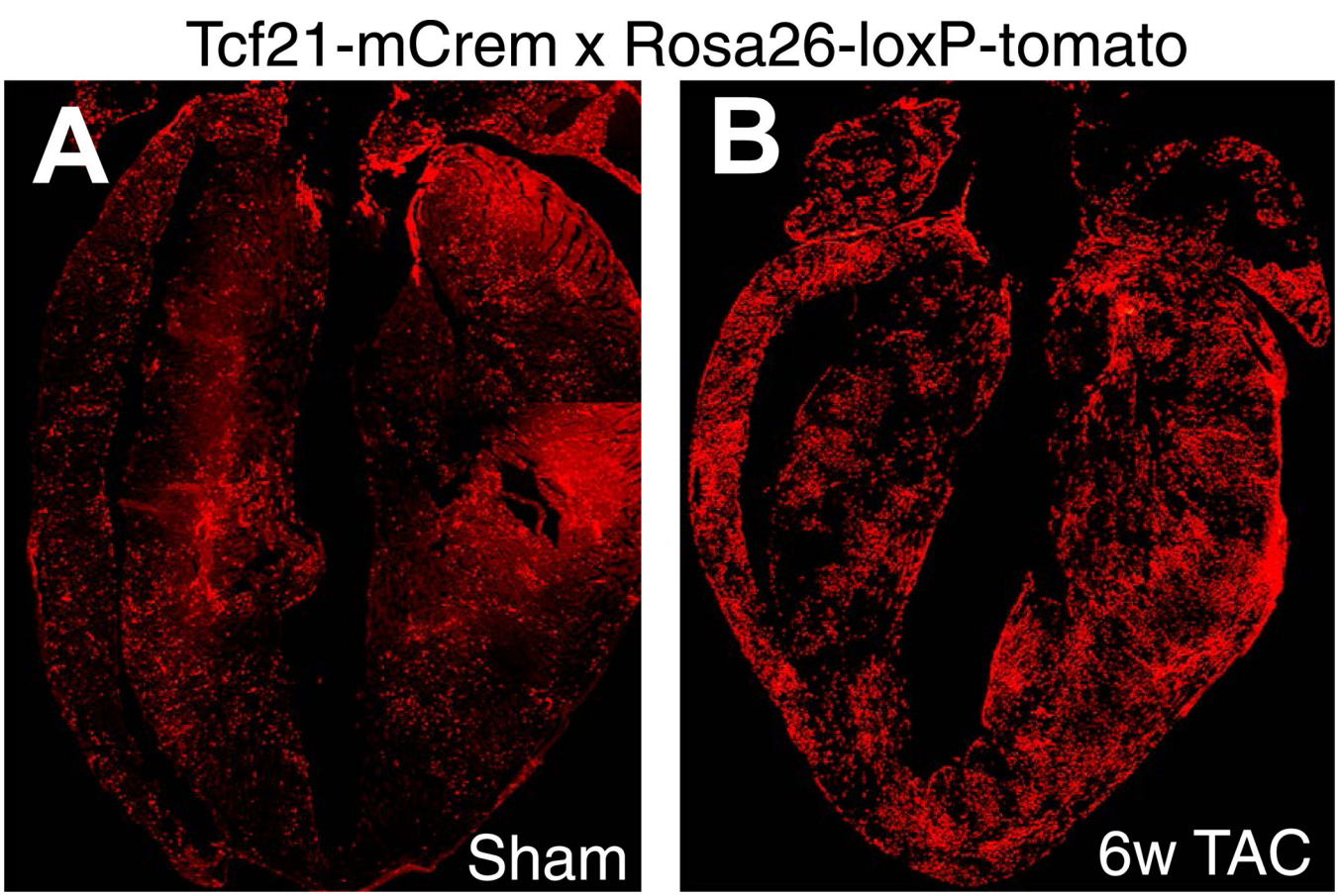

Figure 3 |. Lineage tracing of resident cardiac fibroblasts.

Hearts from adult *Tcf21*^{mCrem} mice containing a Cre-dependent ROSA26^{TdT} indicator that was induced with tamoxifen perinatally. Tissue-resident fibroblasts are labelled red. Mice were subjected to either **a** | a sham surgical procedure (no injury) or **b** | 6 weeks of a thoracic aortic constriction surgical procedure before harvesting. The images show that *Tcf21*-lineage tissue-resident fibroblasts are uniformly distributed throughout the heart and that they expand with pressure-overload stimulation.