A new concept of fibroblast dynamics in post–myocardial infarction remodeling

Thomas Eschenhagen

Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Germany, and German Centre for Cardiovascular Research (DZHK), Partner Site Hamburg/Kiel/Lübeck, Germany.

The identity and function of the fibroblast, a highly prevalent cell type in the heart, have remained poorly defined. Recent faithful genetic lineage–tracing studies revealed that during development, the cardiac fibroblasts are derived from the epicardium and the endothelium, whereas in the adult heart, they constitute the cardiac injury–responsive resident fibroblast. In the current issue of the *JCI*, Molkentin and colleagues decipher the time course and mechanism of the fibroblast in response to myocardial infarction (MI). The model they propose is surprisingly simple and clear. It consists of three major phases. First, fibroblasts in the ischemic area die. Second, surrounding fibroblasts proliferate and migrate into the spaces created by dying cardiomyocytes over a few days. The new fibroblasts in the scar are activated and adopt a smooth muscle actin– and periostin-positive "myofibroblast" phenotype, which appears to last as long as the scar is not mature (~10 days after MI). In the third phase, initially proliferating myofibroblasts lose smooth muscle actin expression and convert to a nonproliferating, matrix-producing phenotype with a newly acquired tendon gene signature. Interestingly, this state appears to differ from that of quiescent fibroblasts in the uninjured heart, as it is resistant to proliferative stimuli. These cells are therefore termed "matrifibrocytes," a novel category whose study will certainly further advance the field.

Difficulty in lineage tracing of fibroblasts in development and disease

The heart is a muscular pump that provides the organism with pulsatile perfusion by blood. Three major subtypes constitute the heart. In humans (1), around 3 billion cardiomyocytes (CMs), the contractile machines of the heart, work in synchrony and in a spatially complex manner to rhythmically expel blood and allow refilling of the heart. Similar numbers of endothelial cells (ECs) are organized in a dense network of capillaries that provide the machines in a 1:1 coupling with the necessary nutrients and oxygen to meet their high-energy demand. The most common cells in the human heart (5–10 billion), however, are cells negative for CM and EC markers and positive for the mesenchymal markers PDGFRB, fibroblast-specific protein 1 (FSP1), and/or smooth muscle actin (SMA) (1). Besides (vascular) smooth muscle cells and pericytes, this population mainly encompasses cells commonly referred to as fibroblasts, cells that synthesize the extracellular matrix and collagen and play a critical role in wound healing. In the heart, fibroblasts and their product, the extracellular matrix, are important for determining part of the passive mechanical properties of the myocardium, e.g., the compliance in diastolic filling, and for stabilizing the heart in case of injury.

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While these principal functions of cardiac fibroblasts are undisputed, details of their biology have remained elusive. One reason is their complex morphology and spatial organization in the heart. While the large rod-shaped CMs are easy to differentiate from other cells just by their shape and ECs exclusively line blood vessels, fibroblasts, on the other hand, are thin, branched cells intimately intermingled with CMs and ECs, making it virtually impossible to differentiate them from neighboring cells via classical light microscopy. A more critical reason is the absence of molecular markers that positively and uniquely identify fibroblasts, which would allow for definitive lineage-tracing experiments to track these cells in vivo. The problem is further aggravated because the functional state, and corresponding molecular signature, of fibroblasts is highly dynamic, causing some markers to be switched on and off depending on the state of the cell. Another important source of error is the lack of specificity of markers or mouse lines used for lineage-tracing experiments, as some markers and genetic lineage–tracing lines label fibroblasts, ECs, and immune cells. Examples of nonspecific fibroblast markers are vimentin (expressed in many cells, including ECs), CD90 (Thy1, expressed also in immune cells, ECs, and pericytes), and FSP1 (also or even primarily expressed in immune cells; see review in ref. 2). On the other hand, Tie1, often used to specifically label ECs, also marks hematopoietic cells (3). Consequently, lineage-tracing experiments using these markers come to different conclusions and lead to a large degree of discrepancy among the definition, developmental origin, and function of cardiac fibroblasts (4).

New lineage-tracing methods answer important questions

Recent work appears to have solved many of the issues discussed above and has

begun to provide a clear picture. Instrumental were three genetic lineage–tracing mouse lines that are now widely accepted as faithful fibroblast reporters for the heart. The first is a transgenic mouse line expressing a GFP reporter under control of the collagen1a1 enhancer (5) used by the Evans group (6), the second is from a mouse in which GFP was inserted into the gene encoding PDGFRα (7), and the third is a line by the Tallquist (8) and the Molkentin groups (9) that uses the transcription factor Tcf21 (epicardin) as a driver. Studies with these and other driver lines answered two important questions: Where do fibroblasts come from during mammalian heart development, and what is the source of fibroblasts in cardiac injury? According to this comprehensive work, fibroblasts derive from the epicardium and the endothelium by epithelial-mesenchymal transition (EMT) and endothelial-mesenchymal transition (EndMT), respectively (2, 6), in development, but predominantly or even exclusively by proliferation of a resident fibroblast population in injury (6, 9). The latter is particularly important, as others have reported that fibroblasts in cardiac injury derive from ECs by EndMT (10), from the epicardium by EMT (11), or from bone marrow cells (12) with different therapeutic consequences. Thus, antifibrotic treatment measures in cardiac diseases should not target EndMT or EMT, but proliferation from resident fibroblasts. It is interesting to note that the difficult path to a universally accepted and likely true interpretation mirrors that in the cardiac field, which finally also came up with a similar, "conservative" conclusion, namely that the few newborn myocytes after injury derive from existing myocytes by cell division (13).

In this issue of the *JCI* (14), the Molkentin group used a comprehensive genetic lineage–tracing approach to precisely follow fibroblasts after myocardial infarction (MI) over time. They crossed a mouse line, in which a tamoxifen-activated Cre recombinase (modified estrogen receptor system; MerCreMer [MCM]) was inserted in the Tcf21 locus, with a line in which GFP was inserted into the ubiquitous *Rosa26* gene locus, flanked by two LoxP sites. In the crossed $Tcf21^{MCM}$;$Rosa26^{GFP}$ line, tamoxifen activates Cre in $Tcf21^+$ cells and marks them permanently as GFP positive. This strategy enabled the authors to label all fibroblasts in the adult heart, bypassing any developmental aspect. One week after labeling fibroblasts with a tamoxifen diet, they induced MI and followed these GFP^+ cells over time and studied their rate of proliferation and expression of marker proteins. This extensive work led to a number of clear conclusions. (a) Not unexpected, but somewhat ignored, is the observation that infarction kills not only the CMs, but also almost all GFP^+ cells (fibroblasts) in the area of ischemia. (b) Three to four days after injury, fibroblasts repopulate the scar and reach a 3.5-fold higher density than in the uninjured state and remain high over at least four weeks. The cells appear to derive from a proliferative burst of fibroblasts in the border zone and, with a delay of one day, in the scar, to which they later migrate. (c) The proliferating fibroblasts in the scar initially adopt a myofibroblast phenotype, indicated by a high percentage of SMA and periostin positivity. (d) Interestingly, however, both proliferation and SMA positivity sharply decline early after injury, as the scar fully forms in the first ten days after injury. Lineage tracing with two additional lines permanently labeling SMA^+ cells ($Acta2^{CreERT2}$;$Rosa26^{GFP}$) and $periostin^+$ cells ($Postn^{MCM}$;$Rosa26^{GFP}$), respectively, showed that these cells (myofibroblasts) do not disappear, but adopt a SMA^- nonproliferative phenotype in the stable scar. (e) Interventions modifying normal collagen maturation by treating with β-aminoproprionitrile or by conditionally deleting periostin (both known to be associated with compromised infarct healing) prolonged the SMA^+ state of fibroblasts, suggesting that the myofibroblast state is lost as soon as the scar matures. (f) Finally, by challenging the mice with a second profibrotic stimulus four weeks after MI (infusion of angiotensin II and phenylephrine), the authors found that fibroblasts in the scar, but not in uninjured regions of the heart, were largely resistant to this stimulus. Instead, the fibroblasts expressed markers of bone, connective tissue, cartilage, and tendon development, leading the authors to introduce a new term for this presumed fibroblast state as the matrifibrocyte. Deleting these cells (and likely others) in the scar by a cryoinjury directed toward the scar worsened cardiac function, suggesting that the matrifibrocyte plays an important role in maintaining the function of the scarred heart.

Taken these results together, the authors propose a new model of fibroblast dynamics in the heart after MI. After MI, quiescent $Tcf21^+$ fibroblasts residing in the interstitial space between CMs and ECs in the border zone showed a short burst of proliferation and migrated into the space left by dead CMs. In the scar, the cells continued to proliferate for a few days and adopted a transient myofibroblast (SMA^+, $periostin^+$) phenotype that is necessary to support structural integrity and generate a mature collagen-rich scar. After approximately 10 days, the cells stopped proliferating and lost SMA expression, while simultaneously adopting a new state characterized by resistance to apoptosis and a tendon-like gene program. Importantly, these same features were identified in scarred human hearts where such phenotypic features might also function to stabilize the scar.

Summary and future directions

The design of this extensive, data-rich study is straightforward, and conclusions are founded by a multitude of genetic lineage–tracing approaches, experimental interventions, and analyses. While some aspects of this study have been described previously, the present results are important, as they provide a comprehensive picture of fibroblast functions in post-MI remodeling and are based on unequivocal genetic approaches. Of course, open questions remain. Is the "activated fibroblast" a true intermediate differentiated state, or is it just an early myofibroblast as part of a more fluid continuum? Additionally, it remains unclear how helpful the split in nomenclature is between the "activated fibroblast" and "myofibroblast" as true differentiated states, as they could very well be the same. Along these same lines, "matrifibrocytes" and senescent fibroblasts could also be somewhat similar, particularly in light of a recent publication that described senescent fibroblasts as having an antifibrotic effect in perivascular fibrosis in pressure overload (15). The latter cells stained positive for β-galactosidase and $p21^{CIP1}$ and, distinct from the matrifibrocytes, were SMA^+. Another important question is what happens if matrifibrocytes are specifically deleted or their differentiation prevented. This study will help answer these questions. Irrespective

of the answer, this new model provides a surprisingly clear and simple picture of fibroblast action and function after MI that has the potential to become textbook knowledge.

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Address correspondence to: Thomas Eschenhagen, Institute of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany. Phone: 49.40.74105.2180; Email: t.eschenhagen@uke.de.

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