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## What's in a name?

### On fibroblast phenotype and nomenclature

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**Abstract**

Fibroblasts have long been recognized as important stromal cells, playing key roles in synthesizing and maintaining the extracellular matrix, but historically were treated as a relatively uniform cell type. Studies in recent years have revealed a surprising level of heterogeneity of fibroblasts across tissues, and even within organs such as the skin and heart. This heterogeneity may have functional consequences, including during stress and disease. While the field has moved forward quickly to begin to address the scientific import of this heterogeneity, the descriptive language used for these cells has not kept pace, particularly when considering the phenotype changes that occur as fibroblasts convert to myofibroblasts in response to injury. We discuss here the nature and sources of the heterogeneity of fibroblasts, and review how our understanding of the complexity of the fibroblast to myofibroblast phenotype conversion has changed with increasing scrutiny. We propose that the time is opportune to re-evaluate how we name and describe these cells, particularly as they transition to myofibroblasts through discrete stages. A standardized nomenclature is essential to address the confusion that currently exists in the literature as to the usage of terms like myofibroblast, and the description of fibroblast phenotype changes in disease.

Keywords: fibroblast, myofibroblast, phenotype conversion, heart, dermis, pressure overload, infarction, scar

## Introduction

As the primary stromal cells in the body, fibroblasts are – or rather they make – the “glue” that holds everything together. These ubiquitous and typically numerous cells contribute materially to the structure of nearly every tissue in the body, both as key cellular constituents and as synthesizers of the extracellular matrix (ECM), which is comprised primarily of mechanically strong proteins such as collagen to provide structure and resilience while balancing compliance. Yet despite the superficially similar role played by fibroblasts in different tissues, the developmental origins of fibroblasts are highly varied, which contributes to a significant heterogeneity in fibroblast phenotype.

When tissues undergo physical stress or damage, fibroblasts become activated and undergo a series of phenotype changes, initially increasing their ability to proliferate and migrate, then eventually converting fully to myofibroblasts, which greatly reduce their migration and proliferation while simultaneously increasing their synthesis of ECM dramatically (Darby et al. 2014; Ma et al. 2017; Roche et al. 2015). Upon resolution of the stress or damage, myofibroblasts may undergo apoptosis, returning the tissue more or less to its original state, however if resolution is delayed or impeded, myofibroblasts may persist for years, such as may occur after myocardial infarction (van den Borne et al. 2010; Willems et al. 1994). Furthermore, the phenotype of these persistent myofibroblasts may be different than that which arises during the initial response to injury (Czubryt 2012; Ruiz-Villalba et al. 2015). The conversion of fibroblasts to myofibroblasts can thus be either highly beneficial, as in the case of normal wound healing or after myocardial infarction where stable scar formation is required, or detrimental when persistent myofibroblasts lead to long-term scarring and fibrosis, which in the myocardium can contribute to arrhythmogenesis (Czubryt 2012; Hermans et al. 2016).

The complex nature of fibroblast to myofibroblast conversion, coupled with the varied developmental origins of fibroblasts, has led to confusion in the literature around the use of the term “myofibroblast” as well as the description of the phenotype change itself – which has been variously described as activation, conversion or even differentiation. An intermediate phenotype – the proto-myofibroblast – has also been described: an activated fibroblast not yet expressing  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), a hallmark of the myofibroblast phenotype (Tomasek et al. 2002). Without doubt, the fact that myofibroblasts may arise from other cell types, including pericytes, hepatic stellate cells, epithelial cells and endothelial cells, has further added to the confusion around naming conventions. Here we discuss the terminology and nomenclature of both the cells involved and the conversion process itself, with a focus on the myocardium, in order to prompt discussion by the scientific community on the importance of developing unified language for the discussion, study and analysis of these important cells.

### **Fibroblast origins and phenotypes**

Given the significant burden of fibrotic diseases on human clinical outcomes, the past decade has seen a dramatic resurgence of interest in fibroblast and myofibroblast biology (Nanthakumar et al. 2015). The conversion of fibroblasts to myofibroblasts is a critical step in the development of fibrosis, and thus represents an important potential point of attack for therapeutic development (Roche and Czubryt 2015). However, it is important for researchers to employ well-defined terminology for describing fibroblasts, myofibroblasts and their interconversion in order to avoid sowing confusion, drawing inaccurate conclusions, or making unwarranted assumptions. A reasonable starting point is to consider the heterogeneity of

fibroblasts both between and within tissues, and to examine the universal use of the term “fibroblast” itself.

Multiple lineage tracing studies have investigated the developmental origins of fibroblasts within different anatomical sites. Fibroblasts from different tissues within the body display distinct and characteristic transcriptional patterns of gene expression, indicating that fibroblast differentiation varies across tissue types (Chang et al. 2002). Furthermore, even within a single tissue type, heterogeneity of fibroblasts has been reported, likely owing to differing embryonic origins coupled with clonal selection and expansion of fibroblast subsets which may alter response to disease (Jelaska et al. 1999). For example, in the case of dermal fibroblasts, those in facial skin originate from the neural crest, those in the anterior body are derived from the lateral plate mesoderm, and those in the posterior compartment arise from the dermomyotome (Driskell and Watt 2015). Thus, the functional heterogeneity of skin fibroblasts reflects, in part, the existence of different fibroblast lineages, resulting in fibroblasts that may respond differently to environmental stimuli or signals. Following dermal injury, the initial wave of repair that involves collagen deposition and scarring is mediated by a deeply located fibroblast lineage, which when activated expresses myofibroblast markers such as  $\alpha$ -SMA (Driskell et al. 2013). Conversely, a superficial fibroblast lineage is recruited in the re-epithelization that forms hair follicles. The variable contribution of these lineages during healing provides insight into why scars that are rich in ECM may be devoid of hair follicles (Driskell et al. 2013).

The primary cellular source for the cardiac fibroblast population is epithelium-derived. During embryonic cardiac development, cells from the proepicardial organ migrate to constitute the epicardium, a subset of which undergoes epithelial-to-mesenchymal transition (EMT), invades the myocardium and subsequently differentiates into fibroblasts (Lie-Venema et al.

2007; Snider et al. 2009). In contrast, fibroblasts in the atrioventricular valve leaflets and tendinous cords of the mitral and tricuspid valves are primarily derived from the endocardium, through endothelial-to-mesenchymal transition (EndMT) (de Lange et al. 2004; Eisenberg and Markwald 1995). There is evidence that ventricular and atrial fibroblasts, despite apparently common embryonic origins, exhibit key phenotypic differences. Atrial fibroblasts are present in higher density, have higher basal rates of ECM production, and show greater propensity to activation than ventricular fibroblasts – differences that are maintained in the failing heart (Burstein et al. 2008). This same study reported over 200 differences in gene expression between atrial and ventricular fibroblasts. Thus, while embryonic origin can contribute to fibroblast heterogeneity, it is likely that other factors, such as the specific cellular milieu, also have a role to play.

Although no specific protein marker exclusive to fibroblasts has been identified to date, several distinctive markers when considered together can be helpful in identifying organ-specific fibroblasts. In human cardiac tissue, ECM protein markers such as collagen and fibronectin have been used to label and identify fibroblasts. However, these proteins can be also expressed by valve interstitial cells, vascular smooth muscle cells and pericytes (Ivey and Tallquist 2016). The intermediate filament-associated calcium binding protein fibroblast-specific protein 1 (FSP-1) was originally thought to be expressed primarily in cardiac fibroblasts, but further studies have revealed a much broader range of expression, including hematopoietic cells, vascular smooth muscle and endothelial cells in both the non-stressed heart and following pressure overload or myocardial infarction (Kong et al. 2013). Further studies are thus needed to re-evaluate currently-used fibroblast markers, and to evaluate novel putative markers through individual cell transcriptomic and proteomic analysis. The identification of a highly specific fibroblast marker –

whether for fibroblasts in general, or for those specifically in tissues such as the heart – remains a critical priority for the field. However, it is possible that such a specific marker simply does not exist; this problem is compounded by the fact that other cell types such as pericytes and hepatic stellate cells may fulfill fibroblast-like functions while clearly being non-fibroblast in nature, and despite the fact that these cells can contribute to the population of myofibroblasts in pathology (Li et al. 2015).

Ultimately, the relative physiological impact of fibroblast heterogeneity remains unclear, particularly as this heterogeneity may (or may not) impact transition of fibroblasts to myofibroblasts during stress or damage. The phenotype of an individual fibroblast stems from many inter-related factors, including developmental origin, tissue type, and likely many stochastic variables such as dynamic and steady-state physical forces acting on the cell, the specific cellular composition of the milieu, within which the fibroblast exists, and the identity and concentration of growth factors, cytokines and substrates that impinge upon the cell. An important question is thus whether there is anything to be gained by further subdividing fibroblasts into subtypes based on, for example, marker expression. It may also be important to consider the role played by the cell, rather than or in addition to the presence of specific markers, as well as the fate of the cell during pathological stress or damage, i.e. whether the cell converts to a myofibroblast fate. In the heart, an important question is whether all cardiac fibroblasts possess equal potential for converting to myofibroblasts, or even if there are sub-populations differentiable by whether or not they can become myofibroblasts. A related question is whether the same specific population(s) of fibroblasts is activated in different pathologies such as pressure overload versus myocardial infarction.

### **Fibroblast phenotype switching**

Early studies had noted that, following tissue injury, myofibroblasts arising during tissue granulation exhibited distinct morphological characteristics from fibroblasts, such as prominent stress fibers that were hypothesized to contribute to wound contraction (Gabbiani et al. 1971). In the intervening years, many studies have described the physical and functional characteristics of myofibroblasts in various tissue types, and in particular their central role in wound healing and fibrosis. The phenotypic switch from fibroblasts to myofibroblasts represents a major change in cell function brought about by significant changes in gene expression to facilitate the dramatic increase in the synthesis of ECM and related remodeling enzymes such as matrix metalloproteinases (MMPs). Recent work in the field has, however, made it clear that the definition of what constitutes a myofibroblast may need to be revisited – or at a minimum, consideration is needed as to whether additional terms are required to describe what has turned out to be a highly heterogeneous cell population (Ma et al. 2017).

Traditionally, it was believed that fibroblasts are activated to undergo conversion to myofibroblasts – thus, cells were either fibroblasts or myofibroblasts. It was soon realized, however, that other cell states exist. As noted above, the term “proto-myofibroblast” was proposed to describe fibroblasts that had become activated to begin the process of conversion to myofibroblasts but did not yet express  $\alpha$ SMA, a hallmark indicator that fibroblasts had become proper myofibroblasts since stromal fibroblasts do not normally express  $\alpha$ SMA (Tomasek et al. 2002). While it remains unclear whether proto-myofibroblasts actually arise during injury responses *in vivo*, this work has helped to usher in an important discussion on myofibroblast heterogeneity – a discussion that is complicated by variability in wound healing across tissue types.

During skin wound healing, fibroblasts in the vicinity of the injury become activated, resulting in a phenotype that is proliferative and motile; these cells migrate to the site of injury, complete their conversion to myofibroblasts and synthesize a robust ECM to aid in wound closure and repair (Li and Wang 2011). Once the tissue has been repaired, ideally to its original pre-injury state, these myofibroblasts undergo apoptosis, or in some cases adopt a quiescent phenotype, however it remains unclear exactly how these processes are controlled, or what the role of these quiescent cells actually may be (Darby et al. 2014). In some situations, such as diabetes, the cellular and molecular mechanisms governing wound healing may go awry, resulting in wounds that fail to heal, possibly due to a failure of fibroblasts to be fully activated to myofibroblasts (Lerman et al. 2003). Conversely, the persistence of active myofibroblasts at the site of injury may lead to abnormal scarring, including hypertrophic scars or dermal keloids (Sarrazy et al. 2011).

Following cardiac injury, quiescent cardiac fibroblasts may be triggered by growth factors, inflammatory cytokines or physical stress to become activated and undergo phenotype conversion into myofibroblasts (Ma et al. 2017; Roche et al. 2015). Earlier studies had implicated a host of contributory cell types in the genesis of myofibroblasts, including EndMT or EMT of various cell types such as hematopoietic bone marrow cells or pericytes (Al-Hattab et al. 2018; Endo et al. 2007; Kramann et al. 2015; Liu et al. 2012; Zeisberg et al. 2007). However, recent lineage-tracing studies have shown that 80–85% of myofibroblasts derive from resident cardiac fibroblasts within the left ventricle of the adult mouse heart after pressure overload (Ali et al. 2014; Kanisicak et al. 2016). Similarly, resident fibroblasts are the primary contributors to cardiac remodeling after myocardial infarction (Fu et al. 2018). Resident cardiac fibroblasts are

thus the primary source of myofibroblasts during cardiac injury and healing, regardless of the nature of the underlying insult.

Recent studies of post-myocardial infarction healing have led to the recognition of novel phenotypic stages during the conversion of fibroblasts to myofibroblasts and beyond (Fig. 1). Upon tissue injury, fibroblasts mediate inflammatory and immune responses as part of the repair process. Within the first day post-infarction, resident cardiac fibroblasts appear to take on a pro-inflammatory phenotype, releasing several MMPs and inflammatory cytokines and growth factors including IL-1 $\beta$ , IL-6, and TNF $\alpha$  (Ma et al. 2017). Additional secreted factors include pro-fibrotic TGF- $\beta$ <sub>1</sub> and Fibroblast Growth Factors that stimulate further fibroblast migration and activation (Ma et al. 2017). The renin-angiotensin system is also important in fibroblast activation. Not only can angiotensin II stimulate fibroblast activation and collagen synthesis via AT<sub>1</sub> receptors, fibroblasts themselves can generate angiotensin converting enzyme and angiotensin II (Dostal and Baker 1998; Lijnen et al. 2001).

By the third day fibroblasts appear to become anti-inflammatory, assisting in the tissue granulation process by secreting pro-angiogenic and anti-inflammatory factors. During these early days, fibroblasts are proliferative and motile, resembling the proto-myofibroblast state (Fu et al. 2018). Cell phenotype then progresses over days and weeks to that of a more traditional myofibroblast: proliferation and migration decreases while ECM synthesis is sharply increased concomitant with expression of  $\alpha$ -SMA as well as periostin – another marker known to be expressed in myofibroblasts but not fibroblasts (Kanisicak et al. 2016) – leading to formation and eventual maturation of the infarct scar. By approximately four weeks, the majority of periostin-labelled cells undergo apoptosis as evidenced in the presence of TUNEL-positive nuclei (Fu et al. 2018). It has long been known that myofibroblast-like cells remain within the scar years or

decades after the initial insult (Turner and Porter 2013; Willems et al. 1994). Fu *et al.* suggested that the cells persisting longer than 10 days post-infarction are an altered myofibroblast that has lost  $\alpha$ -SMA expression and shows reduced proliferation, which they have termed the “matrifibrocyte” (Fu et al. 2018). Interestingly, these cells showed gene expression patterns reminiscent of chondrocytes and osteoblasts, although distinct from both cell types and from myofibroblasts. It remains unclear if these are the same cells noted in humans years after infarction. It also remains unclear if these cells play specific roles, such as stabilization or maintenance of the scar, or if they are simply remnants that escaped apoptosis.

Another intriguing question is whether matrifibrocytes also appear in other disease processes marked by cardiac fibrosis such as pressure overload. Studies employing genetic lineage tracking of fibroblasts using a periostin-Cre reporter revealed that periostin-expressing myofibroblasts are induced by pressure overload following transverse aortic constriction primarily in the left ventricle free wall and septum, whereas induction of fibrosis by angiotensin II and phenylephrine resulted in fibroblast activation throughout the heart (Kanisicak et al. 2016). However, the question of whether the myofibroblasts arising in this latter study are the same as those arising post-infarction remains to be determined. It also is important to determine how myofibroblasts arise and whether they exhibit a similar life cycle in other tissue types, including those in which myofibroblasts are derived from non-fibroblast populations such as hepatic stellate cells or pericytes.

## Conclusion

Given the clear phenotypic stages occurring as fibroblasts become myofibroblasts (Fig. 1), a question for discussion is whether some of the newly identified stages require individual

names. For example, is it sufficient to use the term “pro-inflammatory fibroblasts” or is another name more appropriate (or necessary)? Clearly, simply using the terms fibroblast and myofibroblast would appear to be overly-simplistic given recent discoveries. One term that has been widely used in the literature is “activated fibroblast” which may be useful for describing cells that are no longer fibroblasts, but which have not yet clearly become myofibroblasts. A caveat to using this term, however, is that it has been used highly variably in the literature to date – including as an alternative description for proto-myofibroblasts and myofibroblasts. A significant consideration is the use of the term fibroblast when myofibroblast is much more appropriate. For example, many commercial suppliers of “fibroblasts” are actually selling myofibroblasts – a fact that is readily determined by assessing expression of  $\alpha$ -SMA and/or periostin – typically due to excessive passaging and extended tissue culture prior to sale. This issue also exists broadly in the literature, with many authors incorrectly referring to their cells as fibroblasts despite clear evidence that phenotype change has occurred, or at least commenced, in their samples.

Another consideration is the nomenclature used to describe the process by which fibroblasts become myofibroblasts. Various terms have been used historically, including activation, phenotype conversion and even differentiation. We propose that activation may be best used to describe the initial phase when fibroblasts take on a proto-myofibroblast-like phenotype, marked by increased proliferation and migration but prior to large-scale ECM synthesis or the expression of myofibroblast markers such as  $\alpha$ -SMA and periostin. In this scheme, phenotype conversion could be used to describe the full conversion of fibroblasts to myofibroblasts, although the presence of multiple phenotypic stages may make this term less desirable. A term such as “myofibrogenesis” may be useful instead. We suggest that the term

“differentiation” should never be used to describe fibroblast to myofibroblast conversion, as it suggests that fibroblasts are not differentiated cells, which is arguably quite inaccurate. The conversion of fibroblasts to myofibroblasts is better considered as a change from one specialized phenotype to another – perhaps a form of trans-differentiation, which may reflect a more accurate description of this process.

No matter which naming conventions are eventually adopted, caution must be employed to ensure that lines are not blurred unnecessarily. Names should be informative. Given the heterogeneity of fibroblasts and myofibroblasts – considering embryonic origin, tissue type and the possibly variable contribution of fibroblast populations to wound healing – there is clearly a need for a broader nomenclature. However, having too many names runs the risk of clouding the literature further than it already is. A symposium to specifically discuss nomenclature for this topic would be timely, given the tremendous current interest in these ubiquitous and multifunctional cells. In discussing the unique and complex biology of these fascinating cells, it is important that researchers all speak the same language.

**Abbreviations**

$\alpha$ -SMA	$\alpha$ -smooth muscle actin
ECM	extracellular matrix
EMT	epithelial-to-mesenchymal transition
EndMT	endothelial-to-mesenchymal transition
MMP	matrix metalloproteinase

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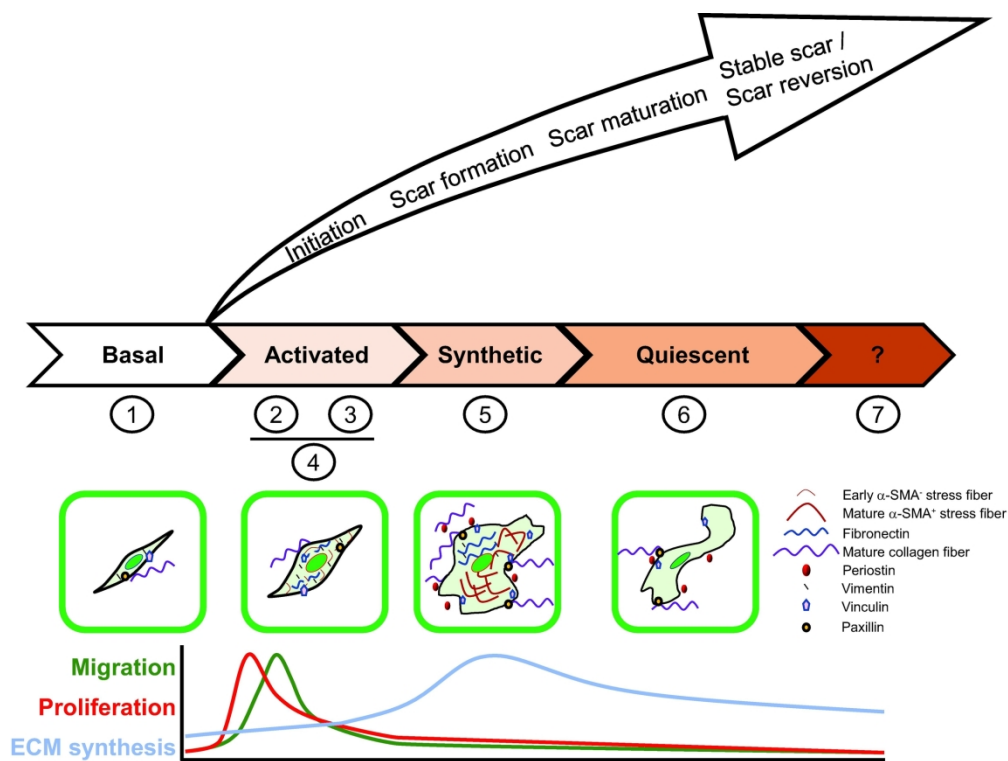
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## Figure Legend

### Figure 1: Putative schema of fibroblast to myofibroblast phenotype conversion.

Basal fibroblasts (1) exhibit relatively low rates of migration, proliferation and synthesis of extracellular matrix (ECM). Upon activation signals such as may occur during tissue damage, e.g. cytokines, growth factors or mechanical stress, fibroblasts initially adopt a pro-inflammatory phenotype (2), increasing their rates of proliferation and migration; this stage is followed shortly by an anti-inflammatory phenotype (3). These early phenotypes, prior to induction of  $\alpha$ -smooth muscle actin expression, may represent the proto-myofibroblast stage (4). Activated fibroblasts decrease their proliferation and migration rates concomitant with a marked increase in ECM synthesis, adopting the synthetic myofibroblast phenotype indicated by expression of markers such as  $\alpha$ -smooth muscle actin and periostin (5). Over time, myofibroblasts reduce ECM production, then either undergo apoptosis, or enter a quiescent phase (matrifibrocyte, (6)) marked by reduced  $\alpha$ -smooth muscle actin expression. It remains unclear what is the exact long-term phenotype of myofibroblasts that persist (7), including whether they maintain the matrifibrocyte phenotype. The long-term persistence of myofibroblasts at the site of injury may determine whether stable scars are formed, or whether the injured tissue fully heals with scar reversion.



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