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Genetic Tools for Identifying and Manipulating Fibroblasts in the Mouse

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Abstract

The use of mouse genetic tools to track and manipulate fibroblasts has provided invaluable in vivo information regarding the activities of these cells. Recently, many new mouse strains have been described for the specific purpose of studying fibroblast behavior. Colorimetric reporter mice and lines expressing Cre are available for the study of fibroblasts in the organs prone to fibrosis, including heart, kidney, liver, lung, and skeletal muscle. In this review we summarize the current state of the models that have been used to define tissue resident fibroblast populations. While these complex genetic reagents provide unique insights into the process of fibrosis, they also require a thorough understanding of the caveats and limitations. Here, we discuss the specificity and efficiency of the available genetic models and briefly describe how they have been used to document the mechanisms of fibrosis.

Keywords

fibroblasts; fibrosis; Cre recombinase; pericytes

Fibrosis

Fibrosis, the deposition of extracellular matrix in response to injury, inflammation, and aging, can be either reparative or reactive. The organs that commonly exhibit fibrosis include the heart, kidney, liver, and lung [1, 2]. Despite the fact that a chronic fibrogenic response ultimately leads to organ dysfunction and failure, accounting for an estimated one third of natural deaths worldwide [1], few therapeutic options have been identified to slow or reverse the detrimental effects of fibrogenesis [1, 2]. Although many forms of injury have been used to induce and study fibrosis in animal models, currently the reagents to trace and assess the cellular and biochemical pathways eliciting the scarring process are limited and complicated. One of the purposes of this review is to describe the reagents that are currently being used to understand fibrogenesis within the mouse.

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Fibroblast definition

One of the key issues to understanding fibrosis is delineation of the role of the fibroblast. This endeavor is complicated, as the fibroblast is poorly defined and sometimes considered immature in regards to its differentiation status [3]. Anatomically, a fibroblast is described as a connective tissue cell that produces extracellular matrix [3]. Within this definition there are two main classifications: the adventitial fibroblast that surrounds blood vessels and the interstitial fibroblast that is not closely associated with any specific structure. The terms mesenchymal and stromal cell are often used interchangeably and indicate a cell present within connective tissue. The production of extracellular matrix is not an apparent requirement for these definitions. Another term requiring definition is pericyte. Originally, a pericyte was described as a cell that shares a basement membrane with an endothelial cell, but this strict, ultra-structural definition has gradually morphed into a more ambiguous definition relying on proximity to a capillary and expression of surface and structural proteins including PDGFR β , NG2, α smooth muscle actin (α SMA), and desmin [4].

Within the field of organ fibrosis, there is considerable controversy over the origin of the cell responsible for reactive fibrosis. These disagreements partially stem from the fact that identification of these cells relies on expression or up regulation of genes including vimentin, collagen, and α SMA (reviewed in [5-7]). Specifically, the term myofibroblast was coined due to a pronounced increase in expression of α SMA in collagen producing cells within injured tissue [8-10]. While a definition based on gene expression is convenient, it restricts identification retrospectively and may represent only a subpopulation of cells. This type of distinction would ignore resident populations of fibroblasts that may be quiescent or not expressing high levels of α SMA. Recent studies using methods to developmentally label fibroblasts raise the issue that α SMA staining underestimates the total population of fibroblasts present after injury [11]. For the purpose of this review, we will routinely use the term “activated fibroblast” to describe the cell populations that respond to injury via proliferation, α SMA expression, and/or collagen production. We will not use the term myofibroblast as it relies heavily on the expression of a single marker.

Adding to the confusion is the fact that many organs have multiple populations of resident mesenchymal cells capable of producing extracellular matrix. These are often identified using different cellular markers, and nomenclature between organ systems is not consistent. Therefore, in this review at the beginning of each organ section, we will outline the cell types considered to have fibroblast-like qualities. Although genetic tools have been used to trace non-resident sources of fibroblasts, especially those arising from bone marrow-derived cells, for the purpose of this review, we will restrict the discussion to tissue resident fibroblast populations within the heart, kidney, liver, lung, and skeletal muscle. For further reading on tools to investigate other sources of fibroblasts the reader is referred to the following studies [12-18].

The purpose of this review is to summarize the current tools available to study the dynamic and enigmatic cell population known as the fibroblast with a focus on the use of genetically engineered mice to identify, follow, and manipulate tissue resident fibroblast populations. We hope that by presenting information for multiple organs, the reader will be able to

identify the most appropriate reagents for their experimental system. Due to the scope of this review we will focus only on resident fibroblast and pericyte populations. For more details on other cell types, please refer to these organ specific reviews that are available for heart [19], kidney [20-22], liver [5], and lung [23].

Fibroblast reporter mouse lines

A key component of tracking a cell population *in vivo* is having a reliable and reproducible method for identifying the cells of interest. Mouse lines expressing reporter genes under the control of transcriptional response elements specific to fibroblasts are a sensitive and efficient way to track these cells. Such reporter genes generally come in two varieties: enzymes with colorimetric substrates or fluorescent proteins. Typically, these lines are generated either by insertional transgenesis (transgenic), where the transgene is injected into the pronucleus of a fertilized oocyte, or by gene targeting (knock-in), where the reporter is introduced in an endogenous gene locus to take advantage of regulatory elements of the host gene. Tables I and II outline available mouse lines that have been used to identify and track fibroblasts in various organs.

Cre indicator/reporter systems

A variation of the direct fibroblast reporter systems outlined above is the use of Cre-driven recombination leading to an indelible and heritable genetic marker of a cell lineage [24]. In the Cre-loxP system, expression of the bacteriophage P1 recombinase, Cre, results in DNA recombination between two loxP sites. These loxP sites can be engineered within specific genomic loci, resulting in gene inactivation after Cre recombination [25]. In the case of Cre indicator mice, expression of a fluorescent or enzymatic protein, such as GFP, tdTomato, or β -galactosidase is induced in the presence of Cre activity, leading to a permanent marking of the cell and its progeny (See Table I for commonly used Cre reporter mice).

These Cre indicator mouse lines have been used to fate map fibroblasts during development and disease processes. The reliability and reproducibility of Cre expression and the sensitivity of reporter detection are important considerations. For example, when using a non-inducible Cre system, Cre activity can occur at unpredictable times or in unexpected cell populations. In these circumstances a sensitive method for identifying Cre activity is required. Prior to the use of Cre reporter mice expressing fluorescent proteins, ROSA26R^{lacZ} [26] mice were routinely used to assess and validate Cre recombination events. While this line has been extremely useful, it is important to remember that detection of β -galactosidase enzymatic activity depends on intact protein. Tissues can be over or under-fixed, resulting in underrepresentation of Cre activity. One way to circumvent this technical limitation is to use an antibody to identify cells that contain β -galactosidase. It should be noted that several tamoxifen-inducible fibroblast Cre lines were generated many years ago when the state of the art Cre reporter was ROSA26R^{lacZ}. Recently, several reports have suggested that this indicator line has reduced sensitivity compared to some of the more recent fluorescent indicator lines [27, 28]. Therefore, reassessment of Cre activity with one of the more sensitive indicator lines is an important experimental consideration. Additionally, some of the newer Cre indicator lines have technical limitations. For example, the commonly used ROSA26R^{mT/mG} indicator line [29] recombines at a significantly lower frequency than

similar indicator lines ([28] and unpublished observations, MDT). In addition, the fluorescent indicators in the ROSA26R^{mT/mG} are reportedly expressed at different levels in various cell types with lung parenchyma having the lowest expression [30]. These ambiguities may have contributed to some of the controversies associated with identification of fibroblast populations during disease.

Additional considerations when using reporters and Cre technology

While the use of Cre technology has provided the unique ability to disrupt gene expression in a relatively controlled manner, there are important methodological controls and caveats that should be considered [31]. One needs to be aware that the genetic manipulation itself may have a phenotype independent of Cre recombination. For example, insertion of loxP sites and vector sequences into a gene locus can result in attenuated gene expression or disruption of nearby genes [32]. Another potential issue is that the Cre mouse line can be compromised. When Cre recombinase is targeted to a specific locus, often a null allele is generated that may result in phenotypic differences compared to wild type controls. Alternatively, when transgenic mouse lines are used, the insertion may disrupt neighboring gene expression or transgene expression can be silenced over time. An additional consideration for many non-inducible Cre mouse lines is that Cre expression or activity can occur in gametes, resulting in germ line recombination [33, 34]. For this reason, it is advised to screen for recombination that occurs in gametes and to maintain the Cre-bearing allele in male breeders where germ line transmission of Cre activity is less frequent.

Tamoxifen inducible systems create an added layer of specificity and complexity. The mode of tamoxifen induction can impact the efficiency of Cre recombination. Tamoxifen can be administered by oral gavage, gastric injection, chow, intraperitoneal injection, or topical cream. Efficiency of Cre activity will depend on availability of tamoxifen to the tissue, Cre protein levels, and accessibility of loxP flanked locus for recombination. It should be noted that high doses of tamoxifen in combination with merCremer (mCrem, a modified tamoxifen inducible Cre construct) may cause focal cardiac fibrosis in a strain dependent manner [35]. Additionally, there are several reports that indicate that Cre expression itself can be cytotoxic, and appropriate controls for monitoring this issue should be established [36-39].

In the field of fibrosis biology, many experiments have been performed to examine the origin of the matrix-producing cell, and Cre-loxP technology is often used to trace cells and their descendants. Only an inducible Cre system should be used to truly identify the cell population of origin. Lineage tracing is different from cell marking in that a discrete population of cells is labeled, and only those cells and their descendants are followed with a Cre indicator. A crucial aspect in this process is the reliability of Cre expression. If Cre expression expands to a new cell lineage, a process that may happen in constitutive Cre lines, the fate mapping system is compromised. Detection of this ectopic or “leaky” Cre recombination has become more frequent with the use of efficient and readily detectable Cre indicator mouse lines [40]. As most murine models of fibrosis include tissue damage, it is important to include uninduced controls when performing fate mapping experiments. Many inducible Cre mouse lines depend on estrogen receptor retention in the cytoplasm until association with tamoxifen. The fidelity of this induction relies on heat shock protein

activity. During tissue injury or stress, the potential exists for tamoxifen independent Cre entry into the nucleus.

Despite these caveats, the use of reporter strains and Cre-loxP technology has provided a wealth of information regarding fibroblast origin and activity.

Tissue resident fibroblasts

Cardiac fibroblasts

Similar to fibroblasts in other organs, cardiac fibroblasts are a poorly-defined cell population [41]. Commonly, vimentin, collagen production, CD90, and DDR2 expression have been used to define the quiescent cardiac fibroblast population, while α SMA expression defines the activated fibroblast [6]. Because many of these markers are expressed by other cell types, it has been difficult to generate a genetic tagging system that is specific to cardiac fibroblasts. One established origin of the cardiac fibroblast is epithelial to mesenchymal transition of the mesothelial layer of the heart known as the epicardium [42]. However, recent analyses have documented that a number of cardiac fibroblasts in the right ventricle and ventricular septum arise from an endothelial lineage [11, 43].

Cardiac fibroblast reporter mouse lines—One of the first reporter mouse strains used to identify cardiac fibroblasts was periostin-lacZ [41]. Subsequently, FSP1-GFP (fibroblast specific protein 1, S4100A) has been used to identify and track cardiac fibroblasts [44]. However, a recent report has suggested that FSP1-driven GFP expression may also occur in leukocytes and endothelial cells after pressure overload and myocardial infarction [45]. Recently, two promising fibroblast reporter mouse strains have emerged: PDGFR α ^{GFP} and Col1a1-GFP. PDGFR α ^{GFP} is a knock-in allele of a nuclear GFP into the PDGFR α locus [46]. In the embryonic and adult heart, GFP is expressed in epicardial cells, cardiac fibroblasts, valvular interstitial cells, and some neural crest derivatives [47, 48]. The Col1a1-GFP transgenic mouse line contains a GFP under the control of a 3.2 kb Col1a1 promoter and 1.7 kb enhancer with DNase-I hypersensitivity sites, originally identified due to GFP expression in hepatic stellate cells (HSC) [49] but now is widely accepted as a tissue resident fibroblast reporter (see also reporter sections for kidney, liver, and lung). In the heart, Col1a1-GFP is expressed in epicardial cells, cardiac fibroblasts, and valvular interstitial cells [43, 48].

Cardiac fibroblast Cre mouse lines—A periostin-Cre line was developed to permit Cre recombination in matrix-producing cells of the heart. Periostin is expressed developmentally, becomes down regulated in the adult, and is re-expressed after injury [50]. As Cre expression is controlled by a 3.5 kb periostin promoter, it is technically not an inducible Cre, but rates of recombination after injury are much higher than at baseline. Although not described in detail with regards to other organ expression, this Cre line is not expressed in cardiomyocytes [51, 52]. Because periostin promoter activity has been reported in vascular smooth muscle cells (VSMC) [50], this population may also contain Cre activity. At baseline, use of this line to recombine floxed (flanked by loxP) alleles resulted in a 4% reduction in Klf5 RNA [51] and a 40% reduction in Tbx20 [53]. After heart injury Cre activity is more efficient, with 72% reduction in Klf5 after thoracic aortic constriction and

65% reduction in GSK3 β after myocardial infarction [54]. These data suggest that the constitutive periostin-Cre may be a useful tool to manipulate gene expression in activated cardiac fibroblasts.

Recently, two inducible Cre lines have been described using periostin regulatory elements. The first is a BAC transgenic line containing periostin promoter elements [55]. This line was used to trace and ablate cardiac fibroblasts after LAD ligation and Angiotensin II treatment. Recombination in ventricular cardiac fibroblasts was observed only after injury. Furthermore, when this periostin-CreERT2 was used to ablate cells after injury a reduction in the periostin-expressing cells was observed. Staining suggested that recombination occurred in over 80% of the cells that were expressing detectable levels of periostin, but no details were provided regarding the number of resident fibroblasts that were not traced and remained after ablation [55]. The second recently described line used targeted mutagenesis to insert mCrem at the periostin translational start site [56]. No Cre activity was observed in induced, uninjured tissues but recombination occurred after injury to heart, lung, skeletal muscle, and skin. Greater than 95% of the traced cells expressed collagen as detected by the Col1a1-GFP line described above and expressed genes important for extracellular matrix production. Cre mediated ablation after LAD ligation resulted in reduced survival rates [56] suggesting that differences may exist between these two periostin-inducible Cre lines. Nonetheless, they appear to be a very promising avenue for inducing recombination in activated fibroblasts.

As collagen genes are commonly expressed by fibroblasts, multiple transgenic mouse lines have been constructed using cis-regulatory elements of a variety of collagen loci (see Table II). In the heart, tamoxifen induction of Col1a2-CreERT^T [57] was used to conditionally delete GSK3 β and resulted in a 60% reduction of protein [54]. This same Cre mouse line was recently used to trace cardiac fibroblasts after injury. The major finding from this study was that some of the lineage-traced cells adopted endothelial gene expression profiles and characteristics [58].

A majority of cardiac fibroblasts derive from the embryonic epicardium by epithelial to mesenchymal transition, and several studies have demonstrated that postnatal epicardial cells produce cardiac fibroblasts [59-61]. Cre lines using Tbx18 and Wt1 cis-regulatory elements have been used to trace this epicardial population. Because the epicardium is the common progenitor of VSMC and cardiac fibroblasts [43, 47, 61, 62], Cre activity occurs in both cell populations. In addition, Tbx18^{CreERT2} and WT1^{CreERT2} do not exhibit extensive recombination in adult cardiac fibroblasts [11, 43]. More problematic is the fact that some of the regulatory elements directing Cre expression (Tbx18 and Wt1) are active in other heart cell populations, including cardiomyocytes and endothelial cells [63-65]. While Wt1 promoter-driven Cre lines have been used to identify epicardial contribution to newly formed fibroblasts and activated fibroblasts [66], the inability to label adult fibroblasts and expression of Wt1 by coronary endothelial cells has complicated the interpretation of these results.

Tcf21 is a transcription factor that is expressed in several mesenchymal populations during development [67, 68], and promoter activity continues to be expressed in many tissue

resident fibroblast populations excluding skeletal muscle, adipose, and dermis (Figure 1 and data not shown). While lacZ alleles exist, they have not been used extensively to study cardiac fibroblast populations [67, 68]. By contrast, tamoxifen induction of a Tcf21^{mCrem} knock-in allele efficiently induces Cre recombination in a vast number of resident cardiac fibroblast cells [48]. We estimate that Cre activity occurs in ~70% of the PDGFR α ^{GFP} expressing cells in the heart (Figure 1F). Furthermore, this population proliferates and expands in response to multiple types of cardiac injury (Figure 1J and manuscript in preparation).

One important consideration for many of the established fibroblast Cre lines in the heart (Col1a2-CreER^T, Tcf21^{mCrem}, and several of the Wt1 locus and periostin locus Cre lines) is that they are also expressed in interstitial cells of the aortic and pulmonary valves, AV sulcus, and/or annulus fibrosis [59,69-72]. These additional sites of recombination should be considered especially when investigating embryonic deletion strategies as alterations in the valves can also lead to cardiac phenotypes [73].

In the heart, there has been much interest in converting fibroblasts into cardiomyocytes after injury. Several studies have used genetic tools to follow fibroblast conversion into the cardiomyocyte lineage after transduction with myocardial differentiation factors. Mouse lines that have been used for these investigations include constitutive periostin-Cre [52] and FSP1-Cre [74] lines as well as the inducible Tcf21^{mCrem} [74]

Kidney fibroblasts

In the kidney several populations of resident cells have been implicated as the injury-induced, matrix-producing cells. These include pericytes and resident interstitial fibroblasts. While mesangial cells, a specialized glomerular, perivascular cell plays a role in glomerulosclerosis, it is not considered a fibroblast nor does it appear to play a significant role in tubulointerstitial fibrosis. In the past, there have been few unique identifiers to distinguish the different cell types in the kidney [21, 75, 76]. This lack of specific markers has impeded progress in understanding the process of fibrosis in the kidney, but data using recently established genetic tools in the mouse have begun to provide a deeper understanding of the origin and function of the cells responsible for extracellular matrix deposition in the kidney.

Kidney fibroblast reporter mouse lines—Several transgenic lines have been used to identify resident mesenchymal populations that contribute to fibrosis. One of the original lines used to identify kidney fibroblasts is FSP1-GFP. FSP1 was initially described as a fibroblast specific protein, and this GFP line has been used to trace tissue fibroblasts in several organs as well as fibroblasts derived from bone marrow [77]. Use of this transgenic line to identify kidney fibroblasts has been brought into question as FSP1-GFP⁺ cells are less abundant than predicted based on recent kidney fibroblast studies [75]. Col1a1-GFP [49] expressing cells account for a broad range of mesenchymal cells in the kidney including pericytes, podocytes, quiescent fibroblasts, and activated fibroblasts [78]. PDGFR α ^{GFP} labels a high percentage of kidney interstitial cells as well as a population of cells within the glomerulus (Figure 1G and data not shown). In addition, an α SMA-RFP has been used to

follow activated kidney fibroblasts in real time showing limited expression without injury but increasing 10-fold after injury [79].

Kidney fibroblast/pericyte Cre mouse lines—Using an extended Acta2 regulatory sequence, a series of transgenic animals were generated to genetically manipulate α SMA-expressing activated fibroblasts [79]. α SMA lineage cells were traced before and after kidney damage [80]. Use of another line with thymidine kinase (tk) expression under the control of the α SMA promoter rendered cells susceptible to gancyclovir-induced cell death [81]. After injury, animals expressing this transgene exhibited a 50-60% reduction in α SMA⁺ cells and reduced kidney fibrosis [80]. In the same study, several additional transgenic lines were used to manipulate kidney pericytes. Using NG2 and PDGFR β regulatory elements, mice were generated to individually express either a fluorescent reporter, thymidine kinase, or Cre recombinase (see Table II for details). Although a detailed analysis was not given, transgene expression was largely restricted to large and small vascular structures under non-pathological conditions and expanded after fibrotic injury [80, 82]. When using thymidine kinase expressing transgenes to ablate cell populations, pericytes were reduced by 80% in the NG2-tk line and by 55% in the PDGFR β -tk line [80].

Recently, a PDGFR β -Cre line [83] has been used to examine the role of α V integrin in kidney fibrosis. In this model, Cre recombination was reported to efficiently target cells in perivascular and intraglomerular locations as well as cells in the renal interstitium. In a unilateral ureteral obstruction (UUO) model of fibrosis, the PDGFR β -Cre line was used to delete α V integrin expression and reduced fibrosis was observed. No efficiency of recombination was reported for kidney cells when using this Cre line [84]. Figure 2G illustrates the overlap between Col1a1-GFP expression and the cells with Cre activity in PDGFR β -Cre kidneys.

One means for targeting a specific population of cells is to generate mouse lines where Cre is expressed in a progenitor population. Manipulation of the progenitor population may be more efficient than targeting a mature cell as all of the subsequent progeny should also be affected by the Cre expression. Often cis-regulatory units from transcription factors permit a specific identification of progenitors. FoxD1 is a transcription factor that is expressed in developing metanephric mesenchyme that will become stromal cells, presumably some of which are fibroblasts [85]. Two knock-in Cre lines were established to follow kidney stromal cells. The constitutive Cre allele recombines in multiple kidney stromal populations in the adult kidney, including interstitial cells, mesangial cells, and pericytes [86]. After injury this tagged population was observed to expand and label positively with α SMA. The inducible FoxD1^{CreERT2} allele was also observed to identify a similar population of cells after either UUO or ischemia reperfusion, but the observed efficiency of recombination was only 20% in pericytes. Because FoxD1 is down-regulated at developmental time points, tamoxifen induction can only occur around embryonic day 10.5 [86]. Subsequent delivery of term pregnancies is often problematic after tamoxifen administration, hampering the utility of this line for temporal deletions. Although FoxD1 provides a system for following the mesenchymal populations, issues with efficiency and specificity could complicate its use for gene deletion.

Tcf21 is an additional gene that is expressed early in kidney mesenchymal cells during development. lacZ [67, 68], Cre [87], and mCrem [48] alleles have been inserted into the Tcf21 locus. Adult induction of the Tcf21^{mCrem} results in robust labeling of a large number of interstitial cells with extensive overlap with PDGFR α ^{GFP} and Col1a1-GFP expressing cells (Figure 1B, G, and data not shown). The ability to temporally delete genes using this system could be a valuable asset to avoid embryonic phenotypes caused by early developmental complications. However, expression of this Cre in podocytes may be problematic when studying the kidney.

Recently, Neelisetty et al. have investigated the role of TGF β receptors in kidney fibrosis using two tamoxifen inducible Cre mouse lines, Col1a2-CreER^T [57] and tenascin^{CreERT2} [88]. Cre reporter positive cells were detected in uninjured animals with low frequencies in the medulla and sparse recombination was observed in the cortex. A population of glomerular cells in the Col1a2-CreER^T kidneys did exhibit recombination. After UUO injury, lineage traced cells from both lines expanded, but the Col1a2-CreER^T animals contained more tagged cells. Only 40% of these tagged cells were also positive for α SMA. Efficiency of gene deletion was only evaluated in cells with Cre reporter activity, thus an overall determination of the efficiency of TGF β deletion was not determined [89].

Liver fibroblasts

As in other organs, there is some debate regarding the population of cells responsible for liver fibrosis. The two cell types implicated are the portal fibroblasts and hepatic stellate cells (HSC). Both cell types can proliferate and express collagen, but relative contributions to fibrosis from each of these cell populations depend on the injury model being investigated [5].

Liver fibroblast reporter mouse lines—The Collagen1a1-GFP line [49] that has been used to follow heart and kidney fibroblasts is not expressed at appreciable levels in adult HSC but is found in embryonic and perinatal HSCs as well as adventitial fibroblasts. However, after CCl₄ induced liver injury, a majority (>92%) of fibroblasts are GFP⁺ [90]. In a bile duct ligation model, Col1a1-GFP transgenic animals were used to follow activated fibroblasts, and it was observed that the GFP⁺ cells were more likely to be portal fibroblasts in this model than HSC (determined by the lack of vitamin A) [91]. Mice expressing fluorescent markers that follow PDGF receptor expression may also be useful for following HSCs and activated fibroblasts [84, 92]. It has been suggested that the FSP1-GFP [77] and FSP1-Cre [93] mouse lines are not appropriate for labeling liver fibroblasts after injury, as the traced cells do not possess α SMA or desmin but do express genes consistent with macrophage and Kupffer cell gene expression [94].

Liver fibroblast/pericyte/HSC Cre mouse lines—Use of constitutive Col1a1-Cre [90] and Col1a2-Cre [95] lines results in tracing of HSC and activated fibroblasts. In uninjured livers, Col1a1-Cre tracing labeled ~31% of the desmin expressing cells, while the overlap increased to 94% after two months of CCl₄ treatment. The Col1a2-Cre line was slightly less robust in tracing desmin⁺ cells with only 16-18% overlap with no injury, 67% overlap after ethanol feeding, and 98% after CCl₄ treatment. Additionally, Col1a2-CreER^T [57] was used

to label HSC during CCl₄ injury, and 35% of the HSC were identified to have Cre recombination [90].

HSC are a unique fibroblast population due to their retinoid storage, and a recent mouse line has been engineered to capitalize on this feature. Mederacke et al. described a lecithin-retinol acyltransferase (Lrat)-Cre that recombines in 99% of vitamin A⁺ HSC [96]. They showed that after CCl₄ induced fibrosis, the Lrat-Cre traced population overlapped 94% with α SMA and 96% with Colla1-GFP. This report also investigated the fidelity and efficiency of recombination in hGFAP-Cre [97] and mGFAP-Cre [98] mouse lines, as GFAP is also considered to be a marker of HSC. Similar to a previous report [99], Cre activity was observed in bile ducts and cholangiocytes but not in HSC as defined by vitamin A autofluorescence or Colla1-GFP expression [96].

Another recently described Cre line used a BAC transgene of the vimentin locus to express CreER. Tamoxifen induction of Cre recombination demonstrated minimal Cre activity in quiescent HSC (~5%) but robust labeling of activated fibroblasts after CCl₄ injection. After bile duct ligation, recombined cells were detected but to a lesser extent than observed after CCl₄-induced injury [100].

Asahina et al. has used a WT1^{CreERT2} line [62] to developmentally trace mesothelial cells at embryonic day 12.5 [101]. They found that some portal fibroblasts, smooth muscle cells, adventitial fibroblasts, and HSC arise from a common mesenchymal progenitor. With this specific tracing technique the authors could only label ~24% of the embryonic desmin positive population [101]. As liver Wt1 expression decreases during embryogenesis, this tool would not be useful for adult HSC inductions.

Similar to WT1, Tcf21 is expressed in the mesothelium of the liver during embryogenesis (unpublished observation, MJJ) and in the adult. Adult tamoxifen induction of Tcf21^{mCre} animals [72] using the ROSA26R^{tdT} indicator and PDGFR α ^{GFP} lines to detect Cre activity and fibroblasts, respectively, identifies a pattern of cells consistent with portal fibroblasts and HSC (Figure 1C, H and unpublished observation, MJJ).

A constitutive PDGFR β -Cre has been used to trace HSC and a high degree of overlap with desmin was observed. Upon injury, the PDGFR β -Cre lineage of cells expanded. The same group used this line to delete integrin α V from liver HSC and observed reduced expression of the integrin [84]. However, use of the PDGFR β -Cre [83] should be carefully evaluated. Breeding to different strain backgrounds and/or rederivation of this line may alter Cre expression from the original cell distribution (personal communication, Ralf Adams). PDGFR β lineage cells do not have significant overlap with Colla1-GFP expressing cells in most organs with the exception of the lung (Figure 2). In addition, the ROSA26R^{tdT} Cre indicator [102] demonstrates a high level of Cre activity in coronary endothelial cells ([103] and Figure 2A). Even so, Cre efficiency for gene deletion using this line is mosaic in many tissues (unpublished observation, MDT). An alternative inducible line, PDGFR β -CreERT2, has been generated with a reported efficiency of ~100% in retinal and adipose pericytes [104].

Several other Cre lines have been used to examine HSC, portal fibroblasts, and activated fibroblasts, but the efficiencies of these lines have not been documented. For example, both α SMA-CreERT2 [105] and GFAP-CreERT2 [106] lines have been used to track activated fibroblast transdifferentiation [107]. Finally, Dermo1^{Cre} [108, 109], a knock-in at the Twist2 locus, was observed to recombine in both pericytes and HSC [110].

Lung Fibroblasts

Within the adult murine lung, two matrix producing cell types have been described: fibroblasts and pericytes. The fibroblast population can be further divided into lipofibroblasts, interstitial fibroblasts, and myofibroblasts [111, 112]. While lung biologists often use the terminology myofibroblast to describe a population of resident fibroblasts, to avoid confusion with an activated fibroblast population, we will refer to these cells simply as fibroblasts. Lipofibroblasts are defined mainly by the presence of neutral lipids within storage vesicles as well as expression of adipocyte differentiation-related protein (ADRP), an amphiphilic protein that coats the neutral lipid droplets, and peroxisome proliferator-activated receptor γ (PPAR- γ) [112-116]. Lung fibroblasts produce collagen and express α SMA when activated [112, 117, 118]. Recently, pericytes (and pericyte-like cells) have been implicated as the source of fibroblasts that respond to injury and disease [22, 86, 119, 120]. Similar to the problems outlined for defining other resident fibroblast populations, the definition of a pericyte within the lung is often ambiguous and dynamic [121, 122].

Lung fibroblast reporter mouse lines—Several reporter lines have been identified for lung fibroblasts. Col1a1-GFP [49] is expressed in ~11% of the nucleated cells in the lung, and gene expression profiling of these cells was consistent with a fibroblast gene profile [123]. Another fluorescent mouse line used to identify lung fibroblasts is PDGFR α ^{GFP} [46]. This tag is abundantly expressed in the developing lung mesenchyme and adult lung fibroblasts. Reports suggest that differing levels of GFP can distinguish between lipofibroblasts and proliferating and quiescent fibroblasts [124-128]. This GFP expressing population was also observed to expand after bleomycin treatment [121]. Finally, FSP1-GFP⁺ cells have been found in the interstitial spaces of healthy and metastatic lung [129], and FSP1-GFP⁺ cells expanded with bleomycin-induced fibrosis [130]. As in other organs FSP1-GFP only appears to identify a subset of lung fibroblasts. A combination of fluorescent reporter mice, nestin-GFP [131] and NG2-DsRed [132] has been used to identify type 1 pericytes (collagen producing) in the lung, defined as NG2-DsRed⁺ and nestin-GFP⁻. In a bleomycin injured lung, type 1 pericytes expanded and produced collagen, similar to what is described below in skeletal muscle. By contrast, in other injury models including myocardial infarction and UUO, type 1 pericytes expanded but did not produce detectable amounts of collagen in these organs [133].

Lung fibroblast/pericyte Cre mouse lines—In addition to PDGFR α ^{GFP} mice, several Cre lines have been generated using PDGFR α regulatory elements. A constitutive PDGFR α Cre line labels both smooth muscle cells and fibroblasts [128]. The lineage-tagged cells using this Cre were either α SMA⁺ or ADRP⁺, suggesting expression in both lipofibroblasts and fibroblasts [128]. Using PDGFR α -CreERT2 mice [134] and tamoxifen induction at perinatal time points investigators were able to preferentially label fibroblasts and not

perivascular smooth muscle cells [128]. PDGFR α may not be the only PDGF receptor that indicates cells capable of responding to injury. Cre indicator tracing of PDGFR β -Cre lung cells tagged pericytes but not interstitial cells. This cell population, nonetheless, was shown to contribute to bleomycin-induced injury [84]. Figure 2C, H illustrates activity of Cre recombination in the lungs of a PDGFR β -Cre mouse.

Several Cre mouse lines using WT1 regulatory elements have been used to investigate the relationship between mesothelium and fibroblasts during development. Lineage tracing data from three WT1 Cre mouse lines suggests that some fibroblast populations arise from a WT1 expressing embryonic progenitor, but WT1 is down regulated in adult lung, complicating controlled induction of recombination events after birth [135-139]. Tcf21 is another transcription factor that is expressed in mesothelium and tissue resident fibroblasts. Adult tamoxifen treatment of Tcf21^{mCre} animals [72] results in labeling of a population of interstitial cells that overlaps with PDGFR α ^{GFP}, although not 100% (Figure 1D, I). These data suggest that either this line is useful in recombining in only a fibroblast subpopulation or that tamoxifen induction was not 100% efficient.

Recombination in lung fibroblast populations was observed using Col1a2-CreER^T starting with postnatal day 8 tamoxifen administration, though range of cell type expression and efficiency of recombination was not documented [57]. More recently, this same line was used to generate animals lacking integrin β 8 in adult lung fibroblasts with a high efficiency as determined in isolated, primary lung fibroblasts [140]. Similarly, Notch1, telomerase, and C/EBP β were efficiently removed from lung fibroblasts after 8 days of tamoxifen treatment in adult mice [141-143].

FoxD1 is expressed in mesenchymal cells that enter the lung buds early in development and upon differentiation expression is silenced. Using a FoxD1 constitutive Cre [144], Duffield and colleagues demonstrated that, similar to the Col1a1-GFP⁺ [49] mesenchymal lung population, FoxD1-derived cells comprised 13.5% of the nucleated cells. In the adult, these cells were identified as pericytes or pericyte-like cells. FoxD1 lineage tracing did not encompass all pericytes. Nonetheless, after bleomycin-induced fibrosis, FoxD1 lineage cells were present in fibroblastic foci and produced collagen [123]. Two additional inducible Cre lines that may be useful in manipulating the lung pericytes are FoxJ1-CreERT2 [145] and NG2-CreERT2 [146], although the recombination efficiency in lung pericytes of the latter line was suboptimal [121].

Fibroblast Growth Factor 10 (FGF10) is expressed in lung mesenchyme and lipofibroblast progenitors and is essential for the early stages of bud formation [147, 148]. Fate mapping using an FGF10^{CreERT2} line [149] indicated that a population of FGF10 lineage cells was positive for the lipofibroblast marker ADRP. Embryonically the FGF10 lineage accounts for a minor population of total lung cells (<2%) and only a fraction of these cells express PDGFR α at the time points examined. These results suggest that either FGF10 progenitors do not contribute significantly to the resident lung fibroblast populations or that Cre induction is suboptimal. A population of smooth muscle cells was also labeled [150].

A Tbx4 enhancer was used to generate a Tbx4-rtTA (tetracycline controlled transactivator) mouse line that enables genetic manipulation of lung mesenchyme [151]. Embryonically, this enhancer was expressed in the entire fibroblast population, but prior to E10.5, there was expression in endothelial cells. In the adult, Tbx4 traced cells were positive for pericyte (NG2⁺) and fibroblast (α SMA⁺) markers and were located adjacent to alveolar epithelial or endothelial cells [151]. Subsequent analyses determined that the majority of labeled cells in the adult lung were pericytes (NG2⁺), and a small number of labeled cells were fibroblasts (α SMA⁺ or FSP1⁺) [152].

The Dermo1^{Cre}, also known as Twist2^{Cre}, mouse line [108, 109] has been used to induce recombination in lung mesoderm including PDGFR β ⁺ fibroblasts, smooth muscle cells, and endothelium [153]. Cre activity was also present in lung mesothelium [154]. When used to delete HDAC3, several mesenchymal markers were down regulated in the lung including vimentin, PDGFR α , and PDGFR β . While there were fewer interstitial PDGFR β ⁺ fibroblasts, the deletion did not result in an entire loss of the population [153]. When Alk5 and PDGFR α were deleted using Dermo1^{Cre} a reduction in interstitial and adventitial fibroblasts was reported and efficiency of gene deletion was greater than 70% [155].

Skeletal muscle fibroblasts

In several types of muscular dystrophy, fibrosis is a pathological hallmark [156], and recent studies suggest that resident fibroblasts are essential components in skeletal muscle regeneration [157] and development [158]. The resident fibroblast populations in skeletal muscle have been called multiple names including: type 1 and type 2 pericytes [159], connective tissue fibroblasts [160], mesenchymal progenitors [161], and fibro/adipogenic progenitors [162]. Although defined by different markers, the consensus is that skeletal muscle contains a resident fibroblast population that expands after injury.

Skeletal muscle fibroblast reporter mouse lines—Because relevance and importance of fibroblast cells in skeletal muscle have only recently been studied, genetic tools in the mouse for identification and manipulation of these cells are limited. One line that may be particularly useful is PDGFR α ^{GFP}. This line has been used to follow and quantify fibroblasts after skeletal muscle injury [163]. Additional studies have described a dual marker system for identification of collagen producing pericytes termed type I pericytes. Using the same NG2-DsRed⁺ [132]/nestin-GFP⁺ [131] tracing system described in the lung, a population of skeletal muscle pericytes was observed to produce collagen upon transplantation into aged mice [159].

Skeletal muscle fibroblast/pericyte Cre mouse lines—The transcription factor Tcf4 is expressed in skeletal muscle fibroblasts, and Cre lines have been generated for lineage tracing and gene deletion. Three targeted mutations exist at this locus: two constitutive and one inducible. These lines have differential abilities to recombine in skeletal muscle fibroblasts. The difference in recombination ability was presumably due to presence of a neomycin resistance cassette, which may have resulted in decreased Cre expression. In mice containing the neomycin gene, recombination was observed in 60% of the fibroblasts. A greater number of fibroblasts were labeled in mice with the allele lacking neomycin, but

recombination was also observed in myogenic cells [160]. A tamoxifen inducible CreERT2 was inserted at the Tcf4 locus, and investigators estimated that ~40% of the skeletal muscle fibroblasts contained recombination after multiple doses of tamoxifen [157].

ADAM12, a membrane metalloprotease, is expressed in cells that contribute to skeletal muscle and dermal fibrosis. Two Cre lines exist for manipulating these cell populations. ADAM12-GFP/Cre is a mouse line expressing a constitutive Cre [164], where ROSA26R indicator mice can be used in combination with the transgene expressing GFP to not only lineage trace but also observe cells with active ADAM12 expression. In a cardiotoxin injury model, ~60-70% of activated fibroblasts were derived from the ADAM12 lineage. The second transgenic line uses a similar strategy but employs a tetracycline transactivator to create an inducible Cre system in which a major fraction of the stroma was identified as ADAM12⁺ cells, but the reported efficiency of recombination was less than 100%.

While myotoxic drugs or muscular dystrophy with myositis (MDM) mice are commonly used to investigate skeletal muscle fibrosis, a genetic model resulting in chronic activation of tissue fibroblasts has recently been reported. Mice possessing tamoxifen-inducible activating mutations in PDGFR α demonstrate skeletal muscle fibrosis [165]. In this model, global induction of Cre using ROSA26R^{CreERT2} with a single dose of tamoxifen in adult animals resulted in activation of PDGFR α and fibrosis in abdominal skeletal muscle and several other organs. These results further demonstrate that PDGFR α manipulation is an important area of study in the field of fibroblasts and fibrosis.

Signaling dependent fibroblast lines

Recently, Gli1-expressing cells have been investigated as a potential perivascular cell population that plays a role in tissue fibrosis. First, Gli1^{lacZ} positive cells [166] were shown to localize around vascular structures in multiple organs. Cells traced by a Cre indicator after induction of Gli1^{CreERT2} [167] mice had localization in heart, lung, liver, and kidney. Furthermore, this cell population expanded after injury. Immunohistochemistry and flow cytometry suggested that these cells express markers of mesenchymal stem cells. Finally, using a dual system where Cre recombination leads to diphtheria toxin receptor (Dtr) expression [168], Kramann et al. demonstrated that ablation of the Gli1 lineage of cells results in reduced fibrosis after injury in kidney and heart. The expansion of Gli1 and Gli1 lineage cells in the kidney [169-171] and lung [172] was confirmed through additional studies. The efficiency of recombination in each tissue was not thoroughly examined, but overlap of the Gli1 lineage of cells (tdTomato⁺) was observed with α SMA and 37-60% of the cells were double positive for the lineage tag and α SMA depending on the organ. Because Gli1 is a downstream target of sonic hedgehog (Shh) signaling, it is no surprise that β -galactosidase expressed from the patched1 (Ptch1) locus [173], another Shh responsive gene, also tags interstitial mesenchymal cells of the kidney [171]. Taken together, these data suggest that a resident Shh-responsive cell population resides in a perivascular niche and is a source of fibrogenic cells that respond to injury. One cautionary note is that these Gli1 alleles reflect Shh signaling and therefore, may be expressed in a lineage independent, location sensitive manner. One might expect that only a subpopulation of a cell type may be labeled or possess recombination at any given time.

Concluding remarks

It is clear from existing data that tools for effectively identifying and manipulating tissue resident cells with fibrogenic potential are in their infancy. The lack of a consensus regarding phenotypic markers for these cells under non-pathological conditions stems from the fact that clear designations for these cell types have not been determined. Recent data has refined our ideas relating to the cell types involved and have reinforced the notion that fibrogenic cells in each organ have unique properties to consider. Many of the Cre lines that have been established do not appear to have high efficiency with regards to gene deletion, highlighting a need for new transgenic line generation. Indeed, new mouse reagents in the pipeline include tamoxifen-inducible Cre lines using PDGFR α , PDGFR β , and periostin regulatory elements. In addition, the field should consider the use of inducible lines with greater frequency to improve specificity and accuracy. Inducible systems are not without fault, as dosing, ectopic expression, and mosaic Cre levels are all variables that need to be carefully controlled. Nonetheless, they may provide a higher degree of specificity. The next few years should prove to be an exciting and informative time for fibroblast biology and treatment of organ fibrosis.

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List of abbreviations

αSMA	α smooth muscle actin, ACTA2
αV integrin	alpha V integrin
ADAM12	A Disintegrin And Metalloproteinase 12
ADRP	adipocyte differentiation-related protein
AV	atrioventricular
BAC	bacterial artificial chromosome
CCl4	carbon tetrachloride
CD90	cluster of differentiation 90
Cre	bacteriophage P1 recombinase
DDR2	discoidin domain receptor tyrosine kinase 2
Dtr	diphtheria toxin receptor
ER	estrogen receptor
FGF10	fibroblast growth factor 10
FoxD	forkhead box D1
FoxJ	forkhead box J1

FSP1	fibroblast specific protein 1, S4100A
GFAP	glial fibrillary acidic protein
GFP	green fluorescent protein
GI	gastrointestinal
Gli1	Gli family zinc finger 1
GSK3β	glycogen synthase kinase 3 beta
HDAC3	histone deacetylase 3
hGFAP	human glial fibrillary acidic protein
HSC	hepatic stellate cells
IHC	immunohistochemistry
Kb	kilobase
Klf5	kruppel-like factor 5
lacZ	β -galactosidase gene
LAD	left anterior descending
loxP	locus of cross-over in P1
Lrat	lecithin-retinol acyltransferase
mCrem	merCremer
mGFAP	mouse glial fibrillary acidic protein
MSC	mesenchymal stem cell
Neo	neomycin resistance
NG2	neural/glial antigen 2
PAC	P1-derived artificial chromosome
PDGFRα	platelet-derived growth factor receptor, alpha
PDGFRβ	platelet-derived growth factor receptor, beta
PPARγ	peroxisome proliferator-activated receptor gamma
Ptch1	patched1
RFP	red fluorescent protein
RNA	ribonucleic acid
rtTA/tTA	tetracycline-controlled transactivator

Shh	sonic hedgehog
Tbx4	T-box 4
Tbx18	T-box 18
Tbx20	T-box 20
Tcf4	transcription factor 4
Tcf21	transcription factor 21
TGFβ	transforming growth factor beta
tk	thymidine kinase
UUO	unilateral ureteral obstruction
VSMC	vascular smooth muscle cells
Wt1	Wilms tumor 1

Figure 1. Tcf21 lineage identifies resident tissue fibroblasts

(A-J) Tcf21 lineage traced cells (red). (A-E) DAPI (blue) and (E-I) nuclear PDGFR α^{GFP} (green) in the specified tissues. (J) Collagen1a1-GFP (green) 7 days after myocardial infarction. Arrowheads indicate examples of cells expressing both tdTomato and GFP. (A-I) Adult mice induced by oral gavage with tamoxifen (0.3 mg/g body weight) for 3 days. (J) Single gavage of tamoxifen (0.3 mg/g body weight) 3 days after ligation of left anterior descending artery (LAD). These data demonstrate that Tcf21^{mCrem} activity overlaps significantly with PDGFR α^{GFP} in most organs with the exception of a population in the lung. Note that the tdTomato reporter is cytoplasmic while GFP from the PDGFR α^{GFP} mouse is nuclear. (A, F) Left ventricular free wall of the heart; (B, G) Cortex of the kidney; (C, D) Left lobe of the liver; (D, I) Distal area of left lung; (E) Skeletal muscle of hind limb; I=interstitium of kidney cortex; G=glomerulus; B=bronchiole; A=alveoli; asterisk(*)=Blood vessel.

Genotypes: (A-D) Tcf21^{mCrem/+};ROSA26R^{tdT/tdT} (E-I)

Tcf21^{mCrem/+};ROSA26R^{tdT/tdT};PDGFR $\alpha^{GFP/+}$ (J) Tcf21^{mCrem/+};ROSA26R^{tdT/tdT}; Coll1a1-GFP.

Figure 2. Distribution of PDGFR β -Cre lineage tagged cells and Collagen1a1-GFP expression (A-J) PDGFR β -Cre lineage tracing in organs (postnatal day 7). ROSA26R^{tdT} Cre reporter mice (red) were used to determine PDGFR β -Cre activity. DAPI (blue). (F-J) PDGFR β -Cre lineage-tagged cells (red) and Collagen1a1-GFP expression (green) (postnatal day 7). PDGFR β -Cre is active in perivascular regions of most organs. Note that in the heart PDGFR β -Cre lineage tracing was observed in endothelial cells (arrowheads in F point out representative capillaries). PDGFR β -Cre and collagen1a1-GFP expression did not co-localize to appreciable levels, although some overlap was observed in the lung. (A, F) Left ventricular free wall of the heart; (B) cortex and medulla of the kidney; (C, H) Left lobe of the liver; (D, I) Proximal area of left lung; (E, J) Skeletal muscle(s) of hind limb; (G) Kidney cortex. Abbrev: I=interstitium; G=glomerulus; D=duct; B=bronchiole; asterisk (*)=Blood vessel. Genotype: (A-E) PDGFR β -Cre;ROSA26R^{tdT/+} (F-J) PDGFR β -Cre;ROSA26R^{tdT/+}; Coll1a1GFP.

Table 1

List of Cre Recombination Reporters

Common Name	Insert Type	JAX Number	Reporter Type	Cre Reporter Expression	Notes	Initial Reference
ROSA26R ^{tdT}	ROSA26 locus	007914 007908	Single	tdTomato	Also known as Ai14 Bright fluorescence may cross into other channels Stable	[102]
ROSA26R ^{tdT}	ROSA26 locus	007909 007905	Single	tdTomato	Also known as Ai9 Neo cassette may reduce reporter expression	[102]
ZsGreen	ROSA26 locus	007906	Single	ZsGreen1	Also known as Ai6 Fluorescence confined to cell bodies	[102]
ROSA26R ^{YFP}	ROSA26 locus	006148	Single	eYFP	Weakly fluorescent Useful for flow cytometry Antibody available	[174]
ROSA26R ^{lacZ}	ROSA26 locus	003474 003309	Single	β -galactosidase	Fixation sensitive Antibody available	[26]
ROSA26R ^{NZG}	ROSA26 locus	012429	Dual	β -galactosidase or eGFP	Nuclear β -galactosidase after Cre recombination eGFP after Cre and FLP recombination Can be modified to a Cre-dependent single reporter (R26 ^{ZG}) by germline excision of the nlslacZ cassette	[175]
ROSA26R ^{mTmG}	ROSA26 locus	007576 007676 026862	Dual	eGFP	Fluorescence localized to cell membrane permitting defined cell boundaries Limited immunohistochemistry Tomato not visible in all cell types Variable recombination	[29]
Z/EG	Transgenic	004178 003920	Dual	eGFP	Low level of β -galactosidase expression in liver and lung Breeding issues	[176]
Z/RED	Transgenic	005438	Dual	DsRed ^{mMST}	Homozygous not viable Breeding issues Possibility of fluorescent protein polymer formation	[177]

More than one JAX number has been included for mice available on different strain backgrounds. Abbreviations: eYFP- enhanced yellow fluorescent protein, Neo-neomycin, eGFP-enhanced green fluorescent protein, nls-nuclear localization sequence

Table II

Gene	Insert	Type	Initial Reference	JAX Number	Fibroblast and/or Pericyte Expression	Expression in Other Cell Types
Adam12	GFP/Cre	BAC	[164]	NA	Skeletal and dermal perivascular cells, stromal cells [164]	Schwann cells [164]
α SMA	tTA/Dtr	BAC	[164]	NA	Profibrotic cells in skeletal muscle and dermis [164]	Nonfibrogenic cells [164]
	Cre	Transgene extended α SMA promoter	[80]	NA	Rare quiescent interstitial cells and activated renal interstitial cells [80]	Occasional renal tubular epithelial cells and occasional blood vessels [80]
	CreERT2	BAC	[105]	NA	Activated liver fibroblasts [107]	Vascular and visceral smooth muscle colon, uterus, kidney, aorta, skeletal muscle, cardiomyocytes [105]
	RFP	Transgene 5.2 kb regulatory element	[79]	NA	Intermittent lung, heart, liver, kidney interstitial cells; activated kidney interstitial cells; and intestinal and testicular stromal cells [79]	
Colla1	thymidine kinase	Transgene extended α SMA promoter	[80]	NA	Rare quiescent interstitial cells and activated renal interstitial cells [80]	
	GFP	Transgene 2.3 kb rat promoter	[178]	013134	Bone marrow activated fibroblasts [179]	Bone and tendon [178]
	GFP	Transgene 3.6 kb rat promoter	[178]	017466 017468	Dermal fibroblasts [178] Activated skeletal muscle fibroblasts [163]	Bladder smooth muscle, bone, and tendon [178]
	GFP	Transgene 4.9 kb mouse promoter	[49, 180]	NA	Activated HSC [49] Activated HSC, embryonic HSC [90] Portal fibroblasts [91] Cardiac fibroblasts [43, 48] Interstitial cells and kidney pericytes [78] Adventitial fibroblasts [181] Lung fibroblasts [123]	Mature osteoblasts [182] Uninjured and injured colon [169] Podocytes [78] Spinal cord perivascular fibroblasts [183]
Colla2	Cre	Transgene 4.9 kb mouse promoter	[90]	NA	Quiescent and activated HSC [90]	
	Cre	PAC	[95]	NA	Mesenchymal cells of dermis, liver, intestine, and heart [95] Quiescent and activated HSC [90]	Microglia, bone, esophagus, spleen [95]
FGF10	CreERT/hpAP	Transgene 6 kb mouse enhancer	[57]	016237	Lung and skin fibroblasts [57] Kidney interstitium [89] Cardiac fibroblasts [58] Dermal fibroblasts [184] Peritoneal submesothelial fibroblasts [185] Lung interstitial fibroblasts [140]	Kidney glomerulus, spleen, gut visceral and vascular smooth muscle [57]
	CreERT2/YFP	Knock-in	[149]		Lung lipofibroblast progenitor [149]	Ear, skin, limbs, cecum [149]

Gene	Insert	Type	Initial Reference	JAX Number	Fibroblast and/or Pericyte Expression	Expression in Other Cell Types
FoxD1	GFP/Cre	Knock-in	[144]	012463	Lung parabrachial and vascular smooth muscle [150] Embryonic renal cortical and medullary interstitial cells, mesangial cells, and pericytes [144] Adult stromal cells, pericytes and activated fibroblasts [86]	
	GFP/CreERT2	Knock-in	[144]	012464	Pericytes and fibroblasts [86] Lung interstitial fibroblasts and pericyte/pericyte-like cells [123]	
FoxJ1	CreER	Transgene 1 kb human promoter	[145]	NA	Lung pericyte-like ciliated cells [121]	Lung airway epithelial cells [145]
Fsp1	GFP	Transgene 3.7 kb promoter	[77]	012893	Cardiac fibroblasts [45] Mammary gland stroma [186] Kidney, liver, lung, and heart fibroblasts [77] Scattered lung interstitial fibroblasts [129] Subpopulation of thymic fibroblasts [187]	Leukocytes after heart injury [45] Bone marrow [77] Liver macrophage/Kupfer cells after BDL or CCl4 [94] Endothelial cells after heart injury [44]
	Cre	Transgene 3.7 kb promoter	[93]	NA	Mammary gland stroma [186, 188] Stomach and prostate stromal fibroblasts, [93]	Liver macrophage/Kupfer cells after BDL or CCl4 [94] Heart non-cardiomyocytes [74]
	thymidine kinase	Transgene 3.7 kb promoter	[81]	012902	Subpopulation of thymic fibroblasts [187] Kidney fibroblasts [81]	
GFAP	Cre	Transgene Human promoter	[97]	004600	HSC [97]	Hepatocytes [99] Neurons, glial cells, bile duct, cholangiocytes [97] Neural progenitors [98]
	Cre	Transgene 15 kb mouse promoter	[98]	012886		
	CreERT2	Transgene human promoter	[106]	012849	Activated liver fibroblasts [107]	Differentiated neurons, astrocytes, and oligodendrocytes [106]
Gli1	CreERT2	Knock-in	[167]	007913	Perivascular MSC-like cells in heart, liver, lung, kidney, and bone marrow [168] Bladder stromal cells [189] Bone fibrocartilage [190] Activated kidney fibroblasts [170] Developing and adult lung mesenchyme [191] Lung bronchiolar smooth muscle and fibroblasts [136]	Developing limb mesenchyme [167] Neural stem cells [192] Taste buds [193] Many other tissues
	nlacZ	Knock-in	[166]	008211	Perivascular cells of heart, liver, lung and kidney [168]	Developing limb mesenchyme [167] Many other tissues

Gene	Insert	Type	Initial Reference	JAX Number	Fibroblast and/or Pericyte Expression	Expression in Other Cell Types
Lrat	Cre	BAC	[96]	NA	Activated kidney fibroblasts [169, Fabian, 2012 #259] Pancreas fibroblasts [194]	
Nestin	Cre	Transgene Rat Regulatory element	[195]	019103 003771	HSC [96] Adipose interstitial [103]	CNS and PNS [195] Kidney and somites [196] Adipocytes [103]
NG2	GFP	Transgene 7.6 kb regulatory element	[131]	NA		Neural stem and progenitor cells [131] Nonpericytes [133, 159]
	Cre	Transgene 5 kb promoter	[80]	NA	Kidney pericytes [80]	Vascular structures [80]
	Cre	BAC	[132]	008533		Oligodendrocytes, astrocytes [132]
	CreER	BAC	[146]	008538	Activated liver fibroblasts [107] Sparse lung pericytes [121]	Oligodendrocytes, astrocytes [146] Sparse brain pericytes [197]
	thymidine kinase	Transgene 5 kb promoter	[82]	NA	Kidney pericytes [80]	Tumor pericytes [82]
	YFP	Transgene 5 kb promoter	[82]	NA	Kidney pericytes [80]	Tumor pericytes [82]
	dsRed	BAC	[132]	008241	Type 1 pericytes (in combination with NG2DsRed) [133, 159]	Oligodendrocytes, CNS mural cells [132]
Patched1	nlacZ	Knock-in	[173]	003081	Kidney interstitial [171]	Occasional tubular epithelial cells, glomerular cells, and endothelial cells [171]
Periostin	GFP/Cre	Transgene 3.2 kb promoter	[51]	NA	Fibroblasts after myocardial infarction [54] Fibroblasts after pressure overload [51] Some cardiac fibroblasts [52]	Outflow tract, AV cushions, adrenal medulla and neural crest [198] Mesenchymal stem cells and bone [199] Semilunar valves [70] Endocardial and endothelial cells [52]
	lacZ	Knock-in	[200]	NA	Cardiac fibroblasts and vascular smooth muscle cells [41, 50]	Cardiac valves [50]
	CreERT2	BAC	[55]	NA	Activated cardiac fibroblasts [55]	Cardiac valves [55]
	mCrem	Knock-in	[56]	NA	Activated cardiac, lung, skeletal muscle, and skin fibroblasts [56]	Adipocytes, bone, cardiac valves [unpublished observation]
PDGFR α	nGFP	Knock-in	[46]	007669	Cardiac fibroblasts [47, 48] Liver fibroblasts [92] Dermis [201] Skeletal muscle fibroblasts [163] Coronary adventitial cells [181] Lung lipo- and interstitial fibroblasts [124, 125, 128]	Broad mesodermal distribution [46] Primitive endoderm [202] Oligodendrocytes [203] Neural precursors [204]
	GFP/CreERT2	Knock-in	[205]	NA	Dermal fibroblasts and lung mesenchyme [205]	Brain glia [205]
	CreERT2	PAC	[134]	NA	Lung fibroblasts [128]	Partial recombination in oligodendrocytes precursors [134] Brown fat adipocytes [206, 207]

Gene	Insert	Type	Initial Reference	JAX Number	Fibroblast and/or Pericyte Expression	Expression in Other Cell Types
	CreER	BAC	[208]	018280		NG2 ⁺ progenitors [208] Some early embryonic deletion in mesoderm [unpublished observation]
	Cre	BAC	[209]	013148	Lung lipo- and interstitial fibroblasts [128]	Retinal Müller cells [209]
PDGFR β	GFP	BAC	[84]	NA	HSC [84]	
	Cre	Transgene	[83]	NA	Skin pericytes [83] Kidney pericytes [80] Lung fibroblast and liver stellate cells [210] HSC, lung and kidney fibroblasts [84]	Adipose endothelial cells and adipocytes [103] Cardiac endothelial cells, kidney mesangial cells [Fig. 2] Cardiac lymphatics [210] Venous endothelial cells [211] Adipose tissue [212]
	Cre	Transgene 4.7 kb promoter	[213]	NA	Dermal fibroblasts [213]	Esophageal smooth muscle, skeletal muscle, medial smooth muscle cells of carotid artery, vascular structures of heart, kidney, lung, brain [213] Brain pericytes [197]
	CreERT2	BAC	[104]	NA	Retinal and adipose pericytes [104]	
	thymidine kinase	Transgene 3.5 kb promoter	[82]	NA	Kidney pericytes [80]	Tumor pericytes [82]
	RFP	Transgene 3.5 kb promoter	[82]	NA	Kidney pericytes [80]	Tumor pericytes [82]
Tbx4	rtTA/lacZ	Transgene 5.5 kb lung enhancer	[151]	NA	Lung smooth muscle, pericytes, interstitial and lipofibroblasts [151] Pericytes [152]	Lung endothelial cells embryonically [152]
Tbx18	CreERT2	Knock-in	[43]	NA	Pericytes [43]	Adult epicardium [43]
	Cre	Knock-in	[61]	NA	Embryonically-tagged epicardial fibroblasts and vascular smooth muscle [61, 11]	Cardiomyocytes [61]
Tcf4	GFP/Cre	Knock-in	[160]	NA	Skeletal muscle connective tissue fibroblasts [160]	Myoblasts [160]
Tcf21	Cre	Knock-in	[87]	NA	Metanephric mesenchyme, kidney interstitial stromal cells, and heart and lung mesenchyme [87]	Podocytes, lamina propria and smooth muscle of gi tract, pancreas, adrenal gland, gonads, choroid plexus and facial muscles [87]
	mCrem	Knock-in	[72]	NA	Coronary smooth muscle and cardiac fibroblasts [48] Kidney mesenchymal cells [72] Lung and liver mesenchyme [Fig 1] Cardiac fibroblasts [74]	Mesothelium [48] Atrioventricular valves [72] Podocytes [72] Mesangial cells [72] Cranial muscles [72]
	lacZ	Knock-in	[67]	NA	Kidney and lung stroma [67] Peritubular cells, pericytes, and adventitial cells [214]	Genitourinary tract [67] Podocytes [214]

Gene	Insert	Type	Initial Reference	JAX Number	Fibroblast and/or Pericyte Expression	Expression in Other Cell Types
tenascin C	lacZ	Knock-in	[68]	NA	Kidney stroma [72] Heart fibroblasts [48]	Cerebellum [88]
Tie2	CreERT2/GFP	Knock-in	[88]	NA	Quiescent and activated interstitial cells [89] Renal inner medulla cells [88]	Vascular endothelial cells [215] Hematopoietic cells [216] Endocardial cushions [43]
Twist2	Cre	Transgenic	[215]	008863	Endocardial derived cardiac fibroblasts [11, 43]	Sclerostome condensensing mesenchyme, osteoblast [108] Facial mesenchyme [218] Germ cells [219]
Vimentin	CreER	BAC	[100]	NA	HSC and pericytes [110] Lung mesenchyme [217] Lung mesenchyme; fibroblasts and smooth muscle cells [153, 155] Dermal fibroblasts [108]	Unidentified cells in stomach, intestine, kidney, colon [100]
Wt1	GFP/Cre	Knock-in	[62]	010911	Activated HSC [100] HSC [220] Lung fibroblasts [139]	Cardiomyocytes [62, 221] Endothelial cells [222] Podocytes [223]
	CreERT2	Knock-in	[62]	010912	Peritoneal submesothelial fibroblasts [185] Annulus fibrosis [69] HSC [101] Coronary smooth muscle and cardiac fibroblast [43, 47] Lung bronchiolar and vascular smooth muscle and fibroblasts [136] Activated lung fibroblasts [137]	Peritoneal mesothelium [185] Sparse cardiomyocytes [62]
	GFP/Cre	BAC	[224]	NA	Coronary smooth muscle and cardiac fibroblast [224] Heart interstitial fibroblasts [59] Lung alveolar and vascular smooth muscle [135]	Valve leaflets, cardiomyocytes [59] Lung endothelial cells, tracheal and bronchial cartilage [135] Septal cardiomyocytes [43]
	Cre	YAC	[225]	NA	Interstitial fibroblasts and alveolar smooth muscle [138]	Sparse lung endothelial cells [138]

Abbreviations: AV-atrioventricular, BAC-bacterial artificial chromosome, BDL-bile duct ligation, CC14-carbon tetrachloride, CNS-central nervous system, Dtt-diphtheria toxin receptor, GFP-green fluorescent protein, gr-gastrointestinal, hpAP-human placental alkaline-phosphatase, HSC-hepatic stellate cells, kb-kilobase, MSC-mesenchymal stem cell, PAC- P1-derived artificial chromosome, PNS-peripheral nervous system, RFP-red fluorescent protein, tTA-tetracycline-controlled transactivator, tk-thymidine kinase, UUO-unilateral ureteral obstruction, YAC-yeast artificial chromosome, YFP-yellow fluorescent protein, NA-not available

Note: The descriptions provided for the cells expressing the reporter or Cre are taken from manuscripts, and we recommend the reader refer to the manuscript to determine how the cell population was defined.