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Tracking Adventitial Fibroblast Contribution to Disease:

A Review of Current Methods to Identify Resident Fibroblasts

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Abstract

Cells present in the adventitia, or outermost layer of the blood vessel, contribute to the progression of vascular diseases, such as atherosclerosis, hypertension, and aortic dissection. The adventitial fibroblast of the aorta is the prototypic perivascular fibroblast, but the adventitia is composed of multiple distinct cell populations. Therefore, methods for uniquely identifying the fibroblast are critical for a better understanding of how these cells contribute to disease processes. A popular method for distinguishing adventitial cell types relies on the use of genetic tools in the mouse to trace and manipulate these cells. As reporter and Cre recombinase expressing mice are used more frequently in studies of vascular disease, it is important to outline the advantages and limitations of these genetic tools. The purpose of this review is to provide an overview of the various genetic tools available in the mouse for the study of resident adventitial fibroblasts.

Keywords

adventitia; fibroblast; perivascular

Introduction

The dynamic functions of the adventitia are a recent interest to vascular biology. Constituents of the adventitia contribute to neointimal hyperplasia[1, 2], extracellular matrix (ECM) production and deposition[3], vessel size regulation[4], and immune cell recruitment[5]. Previous studies mainly relied on in vitro cell culture to understand how these cells respond to pathological conditions[6, 7]. While informative, studies focused on the behavior of cells in culture may not accurately represent in vivo responses with regard to timing, severity, and cellular composition. Experimental approaches in the mouse designed to model diseases such as diabetes, aortic aneurysm, and coronary artery disease have added to our understanding of these pathological processes, but attribution of discrete signaling pathways to a given cell type is complicated due to inefficient methods for identifying and tracking these cell lineages. The heterogeneous nature of the adventitia[3] creates complications in distinguishing cells involved in vascular pathogenesis and fibrosis, and in the past

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delineation of cell populations has relied on morphology or expression of cell specific genes. Advances in genetic markers with Cre-driven recombination and cell type specific reporter technology have permitted in vivo examination of vascular cell populations and their progeny, as well as targeted gene deletion in these cells[8]. However, it is clear that relying on expression of a single gene to identify a cell population that can have a diverse range of injury responses may be problematic. This review aims to define the cells that comprise the adventitial compartment with a focus on the resident fibroblast and to characterize the advantages and disadvantages of the genetic models available to target this cell population. Ultimately, we believe that an understanding of the advantages and the limitations of genetic reagents will result in accurate assessment of their contribution to vascular pathology and eventually lead to improved methods.

Defining the adventitia

Categorizing the resident cell populations of a blood vessel is an important step in understanding cellular contribution to vascular development and disease. In the past, some studies have relied on location within the vessel to define these cells. Larger vessels have three distinct layers: the intima, media, and adventitia. The tunica intima or innermost layer is a monolayer of endothelial cells (EC) in direct contact with blood flow. The intima is separated from the media by a basement membrane and in the case of muscular and elastic arteries, an internal elastic lamina is present[9, 10]. The tunica media consists of multiple concentric rings of vascular smooth muscle cells (VSMC), the number of which depends on vessel size[11, 12]. The tunica adventitia or simply adventitia is separated from the media by an external elastic lamina in arteries and is most the complex layer of the blood vessel[13]. Resident adventitial cells have the capacity to respond to external physiologic stress and remodel the vascular wall[14]. It is important to note that the adventitial fibroblasts are not exclusive to the aorta and all large vessels throughout the body have an adventitial layer that may have a slightly different composition of cells[3, 15]. The diverse subset of cells in the adventitia and putative markers for each are described below:

Adventitial cell populations

Fibroblasts—The cell type most commonly associated with the adventitial layer is the fibroblast. These cells are the predominant resident population of the adventitia and are responsible for depositing abundant collagen fibrils around vessels[15]. Few studies have focused on the embryonic origin of these cells but they are believed to derive from local mesenchymal cell populations[16–19]. The fibroblast is also one of the more difficult cell types to define in vivo. This is likely due to variations in gene expression even in a quiescent state which may reflect cellular origin or anatomic location similar to the VSMC[20]. While genes such as FSP-1, DDR2, and Thy-1 have been used to identify fibroblasts, consistent expression by adventitial fibroblasts in vivo is poorly documented[21–23]. Adventitial fibroblasts are sometimes defined by their location because they are generally separated from the more readily recognized VSMC layer by an external elastic lamina[24]. However, the adventitia has multiple mesenchymal cell populations (described below). Designation based on presence outside of the media may oversimplify matters. Similar to interstitial fibroblast populations, activated adventitial fibroblasts proliferate, deposit ECM, and secrete inflammatory cytokines and chemokines[3, 25–27]. This activated fibroblast, often termed a

myofibroblast, can be identified by expression of contractile proteins such as α-smooth muscle actin (αSMA)[28, 29]. One caution is that αSMA is present in VSMC and can even be heterogeneously expressed in activated fibroblasts[30, 31].

Abundant evidence indicates that these resident fibroblasts contribute to vascular remodeling. After pressure overload in the heart, ECM accumulation is readily observed around the coronary arteries[32, 33], and resident fibroblasts are responsible for a majority of the matrix production[34, 35]. Similarly, matrix producing cells in a mouse model of Duchenne's Muscular Dystrophy originated from the coronary adventitia[36]. Moreover, in the atherosclerotic aorta, media-derived VSMC predominate in the neointima[37], but adventitial fibroblasts can infiltrate lesions and contribute to both the neointima and fibrous cap[38–41].

Vascular Progenitors—Another cell population that resides in the adventitia is the vascular progenitor. These cells are of interest because they may participate in vessel repair and regeneration after injury[42]. Multiple classes of vascular progenitors have been identified including EC[43], VSMC[38, 44], and mesenchymal stem cells (MSC)[14, 45, 46]. Specifically characterizing and lineage tracing these progenitors has been difficult because reagents to uniquely distinguish them are limited[42]. For example, stem cell antigen-1 (Sca1) and CD34 have been used to identify progenitor cells in the adventitia of the aorta that can differentiate into VSMC and EC in vitro[2, 38, 44, 47]. Because these markers are also expressed in other cell populations, the use of lineage tracing or reporter mice to understand the roles of these cells in vivo becomes difficult[48, 49]. Adding to the confusion regarding these progenitors is the recent finding that up to 30% of cells identified as $Sca1^+$ VSMC progenitors have transmigrated from the media to the adventitia in the adult aorta[47], suggesting that there might be cellular exchange between these two anatomic locations.

Pericytes—Pericytes are another mesenchymal cell found in the adventitia. These cells are defined by their proximity to capillaries[50–53] and are distinct from adventitial fibroblasts. In addition, to location, pericytes are often defined by expression of PDGFRβ, NG2, and CD146[50, 51, 54–57]. Some studies suggest that pericytes have fibrogenic potential after injury and can express type 1 collagen[54, 58]. Others have suggested that a unique subset of pericytes is capable of producing ECM[53, 55, 59].

Immune/bone marrow derived cells—Although the adventitia is predominantly comprised of mesenchymal cells, a new appreciation for resident immune cells has developed. In mice, resident immune cells have been described within the adventitial layer and in diseased vessels, the adventitia becomes a coordinating center for inflammatory responses[60–63]. One study points to bone marrow derived fibrocytes in an angiotensin (Ang) II hypertension model[64]. However, there has been recent debate over the extent of immune and bone marrow derived cell contribution to the process of ECM production[34, 35, 65, 66]. Because it is beyond the scope of this review, genetic tools to investigate immune cell conversion into a fibrogenic phenotype will not be discussed.

Genetic tools used to identify adventitial fibroblasts

The use of a combination of markers and mouse genetic tools to identify specific cell populations has permitted researchers to examine the function and influence of adventitial fibroblasts on neighboring cells, but these reagents have limitations and may need further refinement and definition. This section describes available genetic tools that have been used to identify and manipulate these adventitial fibroblast cells (Table 1).

Collagen1a1

Because type I collagen production is one identifying feature of a fibroblast, several mouse lines have been generated using type I collagen cis-regulatory elements to track collagen promoter activity[67–70]. Many mice with type I collagen transgenes have not been documented for expression within adventitial populations. However, *Collagen1a1-GFP* transgenic mice that contain a mutated collagen enhancer element[70] express GFP in the adventitia of coronary arteries, aorta, and pulmonary vein[35] but not cardiac $NG2^+$ pericytes[66].

In postnatal livers, *Collagen1a1-GFP* was observed in both HSC and portal vein fibroblasts, but after postnatal day 14, GFP expression was downregulated[8, 70] and negligible in resting adult liver fibroblasts[68–70]. During hepatotoxic (carbon tetrachloride, CCl_4) and cholestatic (bile duct ligation, BDL) liver injury, *Collagen1a1-GFP* was re-expressed in both portal vein fibroblasts and HSC[67, 69] permitting identification of a population of adventitial fibroblasts.

In uninjured kidney, *Collagen1a1-GFP* was expressed in podocytes and perivascular fibroblasts, but not in mesangial cells or VSMC[58]. After UUO injury, a majority of GFP^+ cells overlapped with αSMA indicating *Collagen1a1* promotor activity in activated cells, but perivascular expression was not determined. While use of genetic tools using *Collagen1a1* cis-regulatory elements to identify fibroblasts is logical, these reagents are unlikely to distinguish between perivascular fibroblasts and interstitial fibroblasts. In addition, this collagen reporter has also been observed in podocytes[58], osteoblasts[71], colon fibroblasts[72], and spinal cord perivascular fibroblasts[73]. Because collagen expression has a dynamic range, it may be difficult to generate genetic reagents that consistently and uniformly label fibroblasts in all organs.

Enolase 2

Although enolase 2 (Eno2) is predominantly a neuron specific protein[74], a recent study demonstrated that Cre activity was observed in the adventitia of the ascending, but not descending aorta[75] in an *Eno2-Cre* transgenic mouse line[76] (JAX #006663, 006297, 005938). The lineage traced cells co-localized with reticular fibroblast marker (ER-TR7) but not with a VSMC marker (αSMA). This line was used to conditionally delete the *AT1a receptor* in fibroblasts to study Ang II-induced medial hyperplasia. In response to Ang II infusion, medial thickness was reduced in the ascending aorta, but the efficiency of recombination was not reported[75]. Further validation of Cre recombination efficiency by this line may be necessary to definitively determine if this Cre line is appropriate for further studies of adventitial fibroblasts.

Fibroblast specific protein 1

Three transgenic mouse lines have been generated using the promoter of *Fibroblast specific protein 1* (*FSP1/S100A4*) including a Cre line[77] (JAX #012641), a thymidine kinase line[78] (JAX #012902), and a GFP expressing line[79] (JAX #012893). The Cre expressing line was used to ablate the AT1a receptor and ~80% reduction in *AT1a receptor* transcript was observed in the aortic adventitia. Ang II-induced medial thickness in the ascending aorta was attenuated in these mice[75]. However, recent studies suggest that *FSP1-GFP* is expressed in immune cells[22] and *FSP1-Cre* recombination was observed in liver Kupffer and macrophage cells after injury[80]. Furthermore, FSP1 protein expression was observed in skeletal muscle pericytes[50] and immune infiltrates after cardiac pressure overload[35]. Therefore, experiments using these lines should consider the possibility of *FSP1* promoter expression in other cell populations when interpreting results.

Gli1

The Gli family of transcription factors mediate sonic hedgehog (Shh) signaling[81] and recently, expression of these genes has been described in perivascular progenitor cells with MSC-like qualities (tri-lineage differentiation, PDGFRβ expression, and adhesion to plastic in vitro) in various organs[59]. Using *Gli1*^{CreERT2}[82] (JAX #007913) for cell labeling, *Gli1* lineage cells were localized to the adventitia of large arteries and arterioles, as well as a pericyte niche[59]. The perivascular proximity of these *Gli1* lineage cells was observed in heart, kidney, lung, liver, bone marrow, and muscle. In the heart, *Gli1* lineage cells expanded after Ang II administration and transverse aortic constriction (TAC), and coincided with ECM production and αSMA expression. Ablation of *Gli1* lineage cells attenuated fibrosis and rescued left ventricular function after TAC. Efficiency and reproducibility of recombination with this Cre line was not demonstrated for adventitial cells. This *Gli1* lineage comprised about 0.02% of the cells in the aortic arch adventitia. After wire injury of the femoral artery or during atherosclerosis, the lineage traced cells could be found within the media and neointima[83]. In atherosclerotic mice (*ApoE*$^{-/-}$ on high fat diet) with induced chronic kidney failure, *Gli1* lineage cells were necessary for calcification of the aortic arch[83]. Single cell analysis demonstrated that the Gli1 lineage of cells were heterogeneous in gene expression[83]. Because these cells are heterogeneous and relatively rare in the adventitia, this Cre may not be ideal for gene ablation studies.

In the same study that implicated *Gli1* lineage cells in the heart, *Gli1* lineage cells were found to contribute to kidney, liver, and lung fibrosis. Cells traced by *Gli1*^{CreERT2} were in perivascular regions in uninjured and injured organs[59]. Lineage traced cells were found outside of the endothelial layer and overlapped with PDGFRβ expression but only constituted a small fraction of the PDGFRβ^{+} cells. After injury, Gli1^{+} cells proliferated and many expressed αSMA, indicating that these cells became activated fibroblasts. Similar to what was observed in the heart, genetic ablation of *Gli1* expressing cells reduced kidney fibrosis after UUO injury. Taken together these data suggest that the *Gli1*^{CreERT2} mouse line labels a subpopulation of adventitial cells that are relevant to vascular pathologies, but further validation of Cre recombination and deletion efficiency is required to determine the role *Gli1* lineage cells play during fibrosis and neointima formation. In addition, *Gli1*^{CreERT2}

recombination occurs in cranial sutures[84]; neural stem cells[85]; hair follicle stem cells[86]; lung mesothelial cells[87]; and lung peribronchial and perivascular smooth muscle[88].

Patched-1 and patched-2

Shh is an important developmental morphogen, but recently a greater role for this molecule has been documented in adult tissues[89]. A role for Shh signaling is becoming evident in the adventitia as well. Reporter activity of *patched-1* and *patched-2*, two Shh receptors, has been documented in the adventitia. At postnatal day 2, *patched-1*^{LacZ}[90] (JAX #003081) and *patched-2*^{LacZ}[91] (JAX #005827) mice exhibit robust β-galactosidase activity in the adventitia of all major arteries including the aortic root, thoracic aorta, coronary, intercostal, mesenteric, and femoral arteries[44, 92]. The extent of the cell labeling was not quantified and expression of the reporter was decreased in adult tissues. Because these receptors are downstream targets of Shh signaling and *lacZ* reporters demarcate cells that are receptive to Shh, reporter expression was seen to increase in the presence of active signaling[93]. As Shh signaling declines with age, these lines may have limited utility in labeling resting adventitial cells. In addition, the hedgehog pathway is active in many cell types, and β-galactosidase expression has been observed in kidney epithelial, glomerular[94], duodenal mesenchymal[95], neural[90], lymphatic endothelial[96], lung mesothelial[87], and hair follicle stem cells[86].

PDGFRα

Recent data has demonstrated that PDGFRα is expressed in a wide variety of fibroblast populations including dermal[97], lung[98, 99], liver[100], and cardiac[34, 35, 65, 101–103] fibroblasts. *PDGFRα*^{nGFP} mice[104] (JAX #007669) express a nuclear H2B-eGFP from the *PDGFRα* locus and are a useful tool to identify fibroblasts in a majority of organs. In the heart, cells expressing GFP were observed in the coronary artery, the thoracic aorta adventitia[66], and myocardial interstitium[103]. These cells are not coincident with PDGFRβ expressing cells and are not considered pericytes[66, 101]. In the liver, *PDGFRα*^{nGFP} expression was reported as HSC specific, but after CCl_4 treatment GFP^+ cells accumulated around central and portal veins suggesting that this GFP reporter may also be expressed by portal vein fibroblasts after injury[100]. Lineage traced cells in the skeletal muscle of an inducible *PDGFRα-Cre*^{ER} mouse[105] (JAX #018280) co-localized with collagen production around vessels in both uninjured and injured skeletal muscle[36]. PDGFRα protein and GFP reporter activity are also expressed in a wide variety of cell types including astrocytes[106], neural stem cells[107], oligodendrocytes[108, 109], perichondrium[104] and adipocyte precursors[110]. Thus care should be taken when using these tools as fibroblast specificity is organ dependent and may vary according to the age being studied.

Sca1

Sca1 is a surface receptor that is expressed on many cell types including fibroblasts, hematopoietic stem cells[111], and EC[66]. In *Sca1-GFP* transgenic mice[112] (JAX #012634), GFP^+ cells are observed in the coronary adventitia. These cells were believed to be fibroblast or fibroblasts progenitors, as they were negative for the NG2 pericyte marker[66]. The use of this cell line may be more complicated as bone marrow chimeras suggested that *Sca1-GFP* may also identify a fibrocyte population[64]. Therefore, this reporter line is unlikely

to be useful for general analysis of adventitial fibroblasts as it does not label all of these cells and expression is observed in multiple other cell types[49, 112].

Tcf21

The transcription factor Tcf21 is expressed in adult cardiac fibroblasts and interstitial valve cells[113]. *Tcf21*^{LacZ} reporter mice[114] have expression of β-galactosidase in coronary adventitia, aortic root, and interstitial cells of the heart[115]. In atherosclerotic lesions, β-galactosidase activity was observed on the luminal side of lesions and in the fibrous cap[115]. In the kidney, another *Tcf21*^{LacZ}[116] reporter line showed β-galactosidase activity in adventitial cells[117]. A tool for identifying *Tcf21* lineage cells was generated by inserting an inducible Cre recombinase at the *Tcf21* locus[118] (*Tcf21*^{mCrem}). *Tcf21* lineage cells were present in the adventitia of coronary arteries and the aortic root, as well as aortic root media and fibrous cap after injury[102, 115]. In addition to cells of the heart, adult induction of *Tcf21*^{mCrem} recombination also lineage tags splenic interstitial cells[119], kidney podocytes and mesangial cells, lung interstitial cells, and liver interstitial cells[8, 118]. Although not specifically noted, *Tcf21* lineage cells are observed surrounding arteries in liver, lung, and kidney, but not in the descending aorta (MDT, unpublished observation).

Guidelines for use of lineage markers and Cre lines

Few of the genetic tools described above uniformly label a lineage of cells, or if they do, additional mesenchymal lineages are also marked. To refine fibroblast genetic tools we must first develop ways to distinguish this cell population from other cell types. Although defining these populations has been challenging for many years, new insights into fibrogenic cells are likely to be forthcoming. The use of single cell sequencing can provide additional insights into cell populations and even subgroups within a cell type. Recent single cell analyses have indicated that periostin may be a more robust marker for activated cardiac fibroblasts, but details on adventitial expression were not explored[65, 120]. Because fibroblasts are likely to have a dynamic range of gene expression depending on if they are in a proliferative, inflammatory, anti-inflammatory, or matrix producing phase, it may be useful to focus on genes that are uniformly expressed by fibroblasts such as, PDGFRα or collagens. Another successful tactic used for the cardiac fibroblast has been labelling cells by their developmental origin[34, 35, 102]. While the embryonic origin of some fibroblasts is defined such as the cardiac fibroblasts[16–18], the origin of other adventitial fibroblast populations is still a relative mystery. Hopefully, future studies will investigate this topic.

When using genetic tools, reproducibility and reliability of the reporter or Cre line are imperative. Rigorous details outlining activity of the genetic reagent should accompany all studies. These details should include quantitative evaluations of how consistent the reporter or Cre line is at labeling the cell population of interest and if there is any promiscuity in other cell types. In addition to validating recombination using a Cre reporter allele, efficiency of gene deletion in the cell type should be provided for all studies using Cre lines. For systems that are not inducible, there is the added complication that expression can be acquired by new cell populations after injury, inflammation, or aging. Transplant or adoptive transfer is one method for verification of fidelity, although this procedure might not be

feasible for every circumstance. Potentially, more refined methods for fibroblast identification will help to resolve the questions regarding contribution of fibrocytes, pericytes, and progenitor cells to vascular fibrosis.

Perspectives

The adventitia is not only a gateway between circulation and the surrounding tissues, but in response to vascular injury, the resident adventitial fibroblasts secrete ECM and inflammatory mediators leading to vascular stiffness and tissue disruption[26]. Because regulation of these activities could be beneficial in controlling vascular pathogenesis, the adventitial fibroblast may be an optimal target for therapeutic intervention[24]. It is important to note that some of our current knowledge of adventitial fibroblasts has been extrapolated from studies of general fibroblast responses to injury, and until recently very little information has specifically related to adventitial fibroblasts. As we learn more about the specific and distinct nature of each adventitial cell population, future studies will lead to more refined mouse tools to further our knowledge of vascular fibrosis and tissue regeneration.

Abbreviations

αSMA	α-smooth muscle actin
Ang II	angiotensin II
AT1a	angiotensin II type 1a
BDL	bile duct ligation
CCl_4	carbon tetrachloride
CD34	cluster of differentiation 34/hematopoietic progenitor cell antigen
CD146	cluster of differentiation 146/melanoma cell adhesion molecule
Cre	P1 bacteriophage recombinase enzyme
DDR2	discoidin domain receptor tyrosine kinase 2
EC	endothelial cell
ECM	extracellular matrix
ER-TR7	reticular fibroblasts
Eno2	enolase 2
FSP1/S100A4	fibroblast specific protein 1
GFP	green fluorescent protein

Gli1	Gli family zinc finger 1
HSC	hepatic stellate cell
LacZ	β-galactosidase gene
MSC	mesenchymal stem cell
NG2	neural/glial antigen 2
PDGFRα	platelet derived growth factor receptor α
PDGFRβ	platelet derived growth factor receptor β
PF	portal fibroblast
Shh	sonic hedgehog
Sca1	stem cell antigen-1
TAC	transverse aortic constriction
Tcf21	transcription factor 21
Thy-1/CD90	cluster of differentiation 90
UUO	unilateral ureteral obstruction
VSMC	vascular smooth muscle cell

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Highlights

- Distinguishing the cellular constituents of the adventitia is an important step in understanding the contribution of each cell to vascular diseases, such as hypertension, atherosclerosis, and aortic aneurysm.
- Resident adventitial fibroblasts are main contributors to the disease process that acquire fibrogenic, proliferative, and inflammatory properties after vascular injury.
- This review summarizes the advantages and disadvantages of mouse genetic markers with Cre-driven recombination and cell type specific reporter technology currently available to study adventitial fibroblasts.
- The heterogeneous functions of the adventitial fibroblast warrant additional tools to identify these cells with focus on the adventitia rather than the general fibroblast population to better understand vascular fibrosis and pathogenesis.

Table 1

Genetic tools for adventitial fibroblasts

Perivascular Expression Profile							Expression in other cell types
Mouse line	**JAX #**	**Tissue**	**Cell Type**	**Uninjured**	**Injury/model**	**Reference**	
Collagen1a1-GFP	n/a	Ascending aorta	Adventitial fibroblast	E, P	n/a	35	Interstitial cardiac fibroblasts[35, 102, 103] Activated HSC[68, 121, 122] Embryonic/postnatal HSC[8, 68] Interstitial lung[123] and kidney[58] cells Podocytes[58] Osteoblasts[71] Colon fibroblast[124] Spinal cord perivascular fibroblasts[73]
		Pulmonary vein	Adventitial fibroblast	E, P	n/a	35	
		Heart	Adventitial fibroblast	E, P	TAC	35	
		Heart	Adventitial fibroblast	A	*mdx* mice	66	
		Liver	PF	n/a	CCl_4, BDL	121, 122	
		Liver	PF	E, P	CCl_4	68	
		Liver	PF	P	n/a	8	
		Kidney	Perivascular fibroblast	A	UUO	58	
		Skeletal muscle	Fibro-adipogenic precursors	A	*mdx* mice	36	
Enolase2-Cre	006663	Ascending aorta	Adventitia	A	Ang II	75	Neural cells[75]
FSP/S1004A-Cre	012641	Ascending aorta	Adventitial fibroblast	A	Ang II	75	Liver Kupffer and macrophages[80]
Gli1^{CreERT2}	007913	Ascending aorta	Adventitial MSC-like pericytes	A	*ApoE*$^{-/-}$ mice HFD & CKD	59, 83	Neural stem cells[85] Cranial sutures[84] Hair follicle stem cells[86] Lung mesothelial cells[87] Lung peribronchial and perivascular smooth muscle cells[88]
		Femoral artery	Adventitial MSC-like pericytes	A	Wire injury	83	
		Heart	Adventitial MSC-like pericytes	A	Ang II, TAC	59	
		Liver	Adventitial MSC-like pericytes	A	CCl_4	59	
		Lung	Adventitial MSC-like pericytes	A	Bleomycin	59	
		Kidney	Adventitial MSC-like pericytes	A	UUO, IRI	59	
Patched-1^{lacZ}	003081	Aortic root/thoracic aorta	Adventitia	P	n/a	44	Lung mesothelial cells[87] Hair follicle stem cells[86] Neural tube cells[90] Kidney interstitial, epithelial, glomerular, and endothelial cells[23, 94] Duodenal mesenchymal cells[95] Lymphatic EC[96]
		Heart	Adventitia	P	n/a	44	
		Pulmonary trunk	Adventitia	P	n/a	44	
		Intercostal artery	Adventitia	P	n/a	44	
		Mesenteric artery	Adventitia	P	n/a	44	
		Femoral arteries	Adventitia	P	n/a	44	

Perivascular Expression Profile							Expression in other cell types
Mouse line	**JAX #**	**Tissue**	**Cell Type**	**Uninjured**	**Injury/model**	**Reference**	
Patched-2^{lacZ}	005827	Aortic root/thoracic aorta	Adventitia	P	n/a	44	
		Heart	Adventitia	P	n/a	44	
		Pulmonary trunk	Adventitia	P	n/a	44	
		Intercostal artery	Adventitia	P	n/a	44	
		Mesenteric artery	Adventitia	P	n/a	44	
		Femoral artery	Adventitia	P	n/a	44	
PDGFRα-Cre^{ER}	018280	Skeletal muscle	Perivascular cell	A	CTX	36	Glial progenitors[105]
PDGFRα^{nGFP}	007669	Thoracic aorta	Adventitial cell	A	n/a	66	Interstitial cardiac fibroblasts[35, 101–103] HSC[100] Lung lipofibroblast[98, 99] Dermal fibroblasts[97] Oligodendrocytes[108, 109] Astrocytes[106] Neural stem cells[107] Perichondrium[104] Adipocytes precursors[110]
		Heart	Adventitial cell	A	n/a	66	
		Skeletal muscle	Fibro-adipogenic precursors	A	CTX	36	
		Liver	PF	A	CCl_4	100	
Sca1-GFP	012634	Heart	Adventitia	A	*mdx* mice	66	EC[66] HSC[111]
Tcf21^{lacZ}	n/a	Heart	Adventitial fibroblast	E	n/a	102	Interstitial cardiac fibroblasts[115] Kidney stroma[118]
		Aortic root	Adventitial fibroblast	A	*ApoE*$^{-/-}$ mice HFD	115	
		Heart	Adventitial fibroblast	A	*ApoE*$^{-/-}$ mice HFD	115	
Tcf21^{lacZ}	n/a	Kidney	Adventitial cell	A	n/a	117	Kidney peritubular cells[117] Kidney and lung stroma[116]
Tcf21^{mCrem}	n/a	Heart	Adventitial fibroblast	E, P	n/a	102	Splenic[119], lung, and liver[8] interstitial cellsInterstitial cardiac fibroblasts[102] Kidney podocytes and mesangial cells[118]
		Aortic root	Adventitial fibroblast	A	*ApoE*$^{-/-}$ mice HFD	115	
		Heart	Adventitial fibroblast	A	*ApoE*$^{-/-}$ mice HFD	115	
		Liver	Adventitial fibroblast	A	n/a	MDT, unpublished	
		Lung	Adventitial fibroblast	A	n/a	MDT, unpublished	
		Kidney	Adventitial fibroblast	A	n/a	MDT, unpublished	

Abbreviations: A, adult; Ang II, angiotensin II; $BaCl_2$, barium dichloride; BDL, bile duct ligation; CCl_4, carbon tetrachloride; CKD, chronic kidney disease; CTX, cardiotoxin; E, embryonic; EC, endothelial cell; GFP, green fluorescent protein; HFD, high fat diet; HSC, hepatic stellate cell; IRI, ischemia reperfusion injury; MSC, mesenchymal stem cell; n/a, not available; P, postnatal; PF, portal fibroblast; TAC, transverse aortic constriction; UUO, unilateral ureteral obstruction