ATVB in Focus:
Identification of Vascular Cell Types: Strengths and Weaknesses of Available Cre-Recombinase Mouse Lines

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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. Because lineage tracing relying on Cre-recombinase expressing mice is 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 article is to provide an overview of the various genetic tools available in the mouse for the study of resident adventitial fibroblasts. **(*Arterioscler Thromb Vasc Biol*. 2017;37:1598-1607. DOI: 10.1161/ATVBAHA.117.308199.)**

Key Words: adventitia ■ aorta ■ fibroblast ■ hypertension ■ mice

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] Although 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 mellitus, 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 by 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, delineation of cell populations has relied on morphology or expression of cell-specific genes. Advances in genetic markers using P1 bacteriophage 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 article 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 think 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.

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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 3 distinct layers: the intima, media, and adventitia. The tunica intima or innermost layer is a monolayer of endothelial cells 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 physiological stress and remodel the vascular wall.[14] It is important to note

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Nonstandard Abbreviations and Acronyms	
αSMA	α-smooth muscle actin
Ang II	angiotensin II
Cre	P1 bacteriophage recombinase enzyme
ECM	extracellular matrix
FSP1	fibroblast-specific protein 1
GFP	green fluorescent protein
Gli1	Gli family zinc finger 1
PDGFR	platelet-derived growth factor receptor
Shh	sonic hedgehog
Sca1	stem cell antigen-1
Tcf21	transcription factor 21
VSMC	vascular smooth muscle cell

that 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 thought 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 because of variations in gene expression even in a quiescent state which may reflect cellular origin or anatomic location similar to the VSMC.[20] Although genes, such as *Fsp1* (fibroblast-specific protein 1; *S100a4*), *DDR2* (discoidin domain receptor tyrosine kinase 2), and *Thy1* (thymus cell antigen 1; *CD90*), 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 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 endothelial cells,[43] VSMC,[38,44] and mesenchymal stem cells.[14,45,46] Specifically, characterizing and lineage tracing these progenitors have been difficult because reagents to uniquely distinguish them are limited.[42] For example, stem cell antigen-1 (Sca1) and cluster of differentiation 34 have been used to identify progenitor cells in the adventitia of the aorta that can differentiate into VSMC and endothelial cells 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 concerning 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 2 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.[55] In addition, to location, pericytes are often defined by expression of PDGFR (platelet-derived growth factor receptor) β, neural/glial antigen 2, and cluster of differentiation 146.[50,51,54–57] Some studies suggest that pericytes have fibrogenic potential after injury and can express type 1 collagen.[58] Others have suggested that a unique subset of pericytes is capable of producing ECM.[51,53,59]

Immune/Bone Marrow–Derived Cells

Although the adventitia is predominantly composed 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 co-ordinating center for inflammatory responses.[60–63] One study points to bone marrow–derived fibrocytes in an angiotensin II (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 article, 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

Table. Genetic Tools for Adventitial Fibroblasts

Perivascular Expression Profile							Expression in Other Cell Types
Mouse Line	JAX No.	Tissue	Cell Type	Uninjured	Injury/Model	References	
Collagen1a1-GFP	n/a	Ascending aorta	Adventitial fibroblast	E, P	n/a	35	Interstitial cardiac fibroblasts[35,67,68]; Activated HSC[69–71]; Embryonic/post-natal HSC[8,69]; Interstitial lung[72] and kidney[58] cells; Podocytes[58]; Osteoblasts[73]; Colon fibroblast[109]; Spinal cord perivascular fibroblasts[75]
		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	70,71	
		Liver	PF	E, P	CCl_4	69	
		Liver	PF	P	n/a	8	
		Kidney	Perivascular fibroblast	A	UUO	58	
		Skeletal muscle	Fibro-adipogenic precursors	A	mdx mice	36	
Enolase 2-Cre	006663	Ascending aorta	Adventitia	A	Ang II	76	Neural cells[76]
FSP/S1004A-Cre	012641	Ascending aorta	Adventitial fibroblast	A	Ang II	76	Liver Kupffer and macrophages[77]
Gli1^{CreERT2}	007913	Ascending aorta	Adventitial MSC-like pericytes	A	ApoE$^{-/-}$ mice HFD and CKD	59,78	Neural stem cells[79]; Cranial sutures[80]; Hair follicle stem cells[81]; Lung mesothelial cells[82]; Lung peribronchial and perivascular smooth muscle cells[83]
		Femoral artery	Adventitial MSC-like pericytes	A	Wire injury	78	
		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[82]; Hair follicle stem cells[81]; Neural tube cells[84]; Kidney interstitial, epithelial, glomerular, and endothelial cells[85]; Duodenal mesenchymal cells[86]; Lymphatic EC[87]
		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	
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[88]

(*Continued*)

Table. Continued

Perivascular Expression Profile							Expression in Other Cell Types
Mouse Line	JAX No.	Tissue	Cell Type	Uninjured	Injury/Model	References	
PDGFRα^{nGFP}	007669	Thoracic aorta	Adventitial cell	A	n/a	66	Interstitial cardiac fibroblasts[35,67,68,89] HSC[90] Lung lipofibroblast[91,92] Dermal fibroblasts[93] Oligodendrocytes[94,95] Astrocytes[96] Neural stem cells[97] Perichondrium[98] Adipocytes precursors[99]
		Heart	Adventitial cell	A	n/a	66	
		Skeletal muscle	Fibro-adipogenic precursors	A	CTX	36	
		Liver	PF	A	CCl_4	90	
Sca1-GFP	012634	Heart	Adventitia	A	mdx mice	66	EC[66] HSC[100]
Tcf21^{lacZ}	n/a	Heart	Adventitial fibroblast	E	n/a	67	Interstitial cardiac fibroblasts[101] Kidney stroma[102]
		Aortic root	Adventitial fibroblast	A	ApoE$^{-/-}$ mice HFD	101	
		Heart	Adventitial fibroblast	A	ApoE$^{-/-}$ mice HFD	101	
Tcf21^{lacZ}	n/a	Kidney	Adventitial cell	A	n/a	103	Kidney peritubular cells[103] Kidney and lung stroma[104]
Tcf21^{mCrem}	n/a	Heart	Adventitial fibroblast	E, P	n/a	67	Splenic,[105] lung, and liver[8] interstitial cells Interstitial cardiac fibroblasts[67] Kidney podocytes and mesangial cells[102]
		Aortic root	Adventitial fibroblast	A	ApoE$^{-/-}$ mice HFD	101	
		Heart	Adventitial fibroblast	A	ApoE$^{-/-}$ mice HFD	101	
		Liver	Adventitial fibroblast	A	n/a	M.D. Tallquist, unpublished data	
		Lung	Adventitial fibroblast	A	n/a	M.D. Tallquist, unpublished data	
		Kidney	Adventitial fibroblast	A	n/a	M.D. Tallquist, unpublished data	

A indicates adult; Ang II, angiotensin II; ApoE$^{-/-}$, apolipoprotein E–deficient mice; BDL, bile duct ligation; CCl_4, carbon tetrachloride; CKD, chronic kidney disease; CTX, cardiotoxin; E, embryonic; EC, endothelial cell; FSP, fibroblast-specific protein 1; 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, post-natal; PDGFR, platelet-derived growth factor receptor; PF, portal fibroblast; TAC, transverse aortic constriction; and UUO, unilateral ureteral obstruction.

used to identify and manipulate these adventitial fibroblast cells (Table).

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.[69,106–108] 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[108] express GFP (green fluorescent protein) in the adventitia of coronary arteries, aorta, and pulmonary vein[35] but not cardiac neural/glial antigen 2$^+$ pericytes.[66]

In post-natal livers, *Collagen1a1-GFP* was observed in both hepatic stellate cell and portal vein fibroblasts, but after postnatal day 14, GFP expression was downregulated[8,108] and negligible in resting adult liver fibroblasts.[69,107,108] During hepatotoxic (carbon tetrachloride) and cholestatic (bile duct ligation) liver injury, *Collagen1a1-GFP* was re-expressed in both portal vein fibroblasts and hepatic stellate cells[70,71] 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 unilateral ureteral obstruction injury, a majority of GFP$^+$ cells overlapped with αSMA indicating *Collagen1a1* promoter activity in activated cells, but perivascular expression was not determined. Although 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,[73] colon fibroblasts,[109] and spinal cord perivascular fibroblasts.[75] 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 is predominantly a neuron-specific protein,[110] a recent study demonstrated that Cre activity was observed in the adventitia of the ascending but not descending aorta[76] in an *enolase 2-Cre* transgenic mouse line[111] (JAX no. 006663). The lineage-traced cells colocalized with reticular fibroblast marker (ER-TR7) but not with a VSMC marker (αSMA). This line was used to conditionally delete the angiotensin II type 1a 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.[76] 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 *FSP1* (*S100A4*), including a Cre line[112] (JAX no. 012641), a thymidine kinase line[113] (JAX no. 012902), and a GFP-expressing line[114] (JAX no. 012893). The Cre-expressing line was used to ablate the angiotensin II type 1a receptor, and ≈80% reduction in angiotensin II type 1a receptor transcript was observed in the aortic adventitia. Ang II–induced medial thickness in the ascending aorta was attenuated in these mice.[76] 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.[77] 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.

Gli Family Zinc Finger 1

The Gli family of transcription factors mediate sonic hedgehog (Shh) signaling,[115] and recently, expression of these genes has been described in perivascular progenitor cells with mesenchymal stem cell–like qualities (trilineage differentiation, PDGFRβ expression, and adhesion to plastic in vitro) in various organs.[59] Using *Gli1*^{CreERT2} (JAX no. 007913)[116] for cell labeling, *Gli1* (Gli family zinc finger 1) 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 and coincided with ECM production and αSMA expression. Ablation of *Gli1* lineage cells attenuated fibrosis and rescued left ventricular function after transverse aortic constriction. Efficiency and reproducibility of recombination with this Cre line were not demonstrated for adventitial cells. This *Gli1* lineage comprised ≈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.[78] In atherosclerotic mice (apolipoprotein E–deficient mice on high-fat diet) with induced chronic kidney failure, *Gli1* lineage cells were necessary for calcification of the aortic arch.[78] Single-cell analysis demonstrated that the Gli1 lineage of cells were heterogeneous in gene expression.[78] 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 unilateral ureteral obstruction 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,[80] neural stem cells,[79] hair follicle stem cells,[81] lung mesothelial cells,[82] and lung peribronchial and perivascular smooth muscle.[83]

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.[117] A role for Shh signaling is becoming evident in the adventitia as well. Reporter activity of *patched-1* and *patched-2*, 2 Shh receptors, has been documented in the adventitia. At postnatal day 2, *patched-1*^{LacZ} (JAX no. 003081)[84] and *patched-2*^{LacZ} (JAX no. 005827) mice[118] 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,] 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.[120] Because Shh signaling declines with age, these lines may have limited use 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,[85] duodenal mesenchymal,[86] neural,[84] lymphatic endothelial,[87] lung mesothelial,[82] and hair follicle stem cells.[81]

Platelet-Derived Growth Factor Receptor α

Recent data have demonstrated that PDGFRα is expressed in a wide variety of fibroblast populations, including dermal,[93] lung,[91,92] liver,[90] and cardiac[34,35,65,67,68,89] fibroblasts. *PDGFRα*^{nGFP} mice[98] (JAX no. 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.[35,67,68,89] These cells are not coincident with PDGFRβ expressing cells and are not considered pericytes.[66,89] In the liver, *PDGFRα*^{nGFP} expression was reported as hepatic stellate cell specific, but after carbon tetrachloride treatment, GFP$^+$ cells accumulated around central and portal veins, suggesting that this GFP reporter may also be expressed by portal vein fibroblasts after injury.[90] Lineage-traced cells in the skeletal muscle of an inducible *PDGFRα-Cre*^{ER} mouse[88] (JAX no. 018280) colocalized 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,[96] neural stem cells,[97] oligodendrocytes,[94,95] perichondrium,[98] and adipocyte precursors.[99] Thus, care should be taken when using these tools because fibroblast specificity is organ dependent and may vary according to the age being studied.

Stem Cell Antigen-1

Sca1 is a surface receptor that is expressed on many cell types, including fibroblasts, hematopoietic stem cells,[100] and endothelial cells.[66] In *Sca1-GFP* transgenic mice[121] (JAX no. 012634), GFP$^+$ cells are observed in the coronary adventitia. These cells were thought to be fibroblast or fibroblasts progenitors because they were negative for the neural/glial antigen 2 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 because it does not label all of these cells, and expression is observed in multiple other cell types.[49,121]

Transcription Factor 21

The transcription factor transcription factor 21 (Tcf21) is expressed in adult cardiac fibroblasts and interstitial valve cells.[122] *Tcf21*^{LacZ} reporter mice[123] have expression of β-galactosidase in coronary adventitia, aortic root, and interstitial cells of the heart.[101] In atherosclerotic lesions, β-galactosidase activity was observed on the luminal side of lesions and in the fibrous cap.[101] In the kidney, another *Tcf21*^{LacZ} reporter line[104] showed β-galactosidase activity in adventitial cells.[103] A tool for identifying *Tcf21* lineage cells was generated by inserting an inducible Cre-recombinase at the *Tcf21* locus[102] (*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.[67,101] In addition to cells of the heart, adult induction of *Tcf21*^{mCrem} recombination also lineage tags splenic interstitial cells,[105] kidney podocytes and mesangial cells, lung interstitial cells, and liver interstitial cells.[8,102] Although not specifically noted, *Tcf21* lineage cells are observed surrounding arteries in liver, lung, and kidney but not in the descending aorta (M.D. Tallquist, unpublished data, 2016).

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.[65,124] 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 labeling cells by their developmental origin.[34,35,67] Although the embryonic origin of some fibroblasts, such as cardiac fibroblasts,[16–18] is defined 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 about 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 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.

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Disclosures

None.

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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.
- This article 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.

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