

# Bone-Forming Perivascular Cells: Cellular Heterogeneity and Use for Tissue Repair






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## CONCISE REVIEW

# Bone-forming perivascular cells: Cellular heterogeneity and use for tissue repair

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### Abstract

Mesenchymal progenitor cells are broadly distributed across perivascular niches—an observation conserved between species. One common histologic zone with a high frequency of mesenchymal progenitor cells within mammalian tissues is the *tunica adventitia*, the outer layer of blood vessel walls populated by cells with a fibroblastic morphology. The diversity and functions of (re)generative cells present in this outermost perivascular niche are under intense investigation; we have reviewed herein our current knowledge of adventitial cell potential with a somewhat narrow focus on bone formation. Antigens of interest to functionally segregate adventicytes are discussed, including CD10, CD107a, aldehyde dehydrogenase isoforms, and CD140a, among others. Purified adventicytes (such as CD10<sup>+</sup>, CD107a<sup>low</sup>, and CD140a<sup>+</sup> cells) have stronger osteogenic potential and promote bone formation in vivo. Recent bone tissue engineering applications of adventitial cells are also presented. A better understanding of perivascular progenitor cell subsets may represent a beneficial advance for future efforts in tissue repair and bioengineering.

### KEYWORDS

adipogenesis, adipose stem cell, ALDH, CD10, CD107a, CD140a, exocytosis, LAMP1, mesenchymal stem cell, mesenchymal stromal cell, osteogenesis, perivascular stem cell, tunica adventitia

### Significance statement

Perivascular cells are a heterogeneous population of mesenchymal progenitors. The outermost layer of the vessel wall is a niche with high density and diversity of progenitors, also called adventitial cells. Several antigens including CD10, CD107, CD140a, as well as aldehyde dehydrogenase activity have been identified in adventitial cells showing distinct differentiation potential with implications for bone repair. In particular, adipose tissue holds great promise for bone tissue engineering due to its high content of mesenchymal progenitors including bone-forming subsets. This publication highlights the most relevant basic and translational research of perivascular cell biology with a focus on bone regeneration.

Jiajia Xu, Yiyun Wang, and Mario A. Gomez-Salazar contributed equally to this study.

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## 1 | INTRODUCTION

From her observations of chicken blastoderms within her anatomy laboratory at Johns Hopkins University, Florence Sabin concluded in 1917 that “Angioblasts and later endothelial cells give rise to red blood cells.”<sup>1</sup> This visionary insight into hematopoietic stem cell (HSC) biology, more than 40 years before Till & McCulloch's discovery of blood cell progenitors, received dazzling experimental confirmations from the 1970s,<sup>2</sup> to result in recent years in the thorough anatomic, molecular, and developmental characterization of the “hemogenic endothelium.”<sup>3</sup> Much later in life, endothelial cells contribute to scarring in the infarcted myocardium by transdifferentiating into fibroblasts<sup>4</sup> in a reversible manner.<sup>5</sup> Moreover, the embryonic dorsal aorta and other adult blood vessels host potent skeletal myogenic progenitors,<sup>6</sup> further illustrating the developmental flexibility of some vascular cells. In a teleological perspective, physical association of progenitor cells with blood vessels should permit the ubiquitous dissemination of tissue regenerative potentials. Such a tentative correlation between anatomy and function guided the search for the native origin of mesenchymal stem cells (MSCs), the culture-derived multilineage mesodermal progenitors that can be extracted from all vascularized tissues.<sup>7</sup> Markers expressed by perivascular cells had been detected on cells from the human uterus that give rise to MSC like progenitors.<sup>8</sup> Then, some of us observed that pericytes, the mural cells that ensheath capillaries and microvessels,<sup>9</sup> purified by flow cytometry from all human organs tested produce MSCs in culture,<sup>10</sup> establishing a perivascular distribution for the forerunners of these multipotent cells. Purified pericytes have been used experimentally to engineer blood vessels,<sup>11</sup> and regenerate lung,<sup>12</sup> skeletal muscle,<sup>13</sup> cartilage,<sup>14</sup> ischemic limbs,<sup>15</sup> tendon,<sup>16</sup> and uterus.<sup>17</sup> Besides, pericytes naturally contribute to regenerating Leydig cells in the testis,<sup>18</sup> satellite cells and myofibers in skeletal muscle,<sup>19</sup> white adipocytes,<sup>20</sup> follicular dendritic cells,<sup>21</sup> dental cells,<sup>22</sup> and fibroblasts in multiple tissues.<sup>23,24</sup> Mesodermal lineage potential is also present in the *tunica media* and *tunica adventitia* of larger arteries and veins, where it has been studied in the context of pathologic vascular remodeling.<sup>25,26</sup> In agreement, presumptive MSCs have been described in the human<sup>27</sup> and murine vascular adventitia,<sup>28</sup> allowing to conclude that blood vessels of all sizes are repositories for mesodermal progenitor cells. Quantitatively, the *tunica adventitia* represents a substantial reserve of primitive mesodermal progenitors<sup>29</sup> of undisputable pathophysiologic relevance and possible therapeutic significance. The diversity and functions of (re)generative cells present in this outermost perivascular niche are under intense investigation; we have reviewed herein our current knowledge of adventitial cell potential with respect to bone formation, in culture and in vivo.

## 2 | DIFFERENT CELL TYPES

### 2.1 | The osteoblastogenic potential of perivascular adventitial cells

Adventitial cells (a.k.a. adventicytes), so-named as they lie in the *tunica adventitia* of blood vessels, have a nondescript fibroblastic

morphology and at times appear to be in continuity with fascial connective tissue. Defined as a CD34<sup>+</sup>CD146<sup>-</sup>Lin<sup>-</sup> cell population, we and others have described their multipotency.<sup>27,28</sup> The osteoblastic potential of adventitial cells has been summarized in several recent reviews.<sup>30,31</sup> Perivascular adventitial cells participate directly in bone formation and repair<sup>32,33</sup> as well as indirectly induce bone repair via interaction with native skeletal cells.<sup>34,35</sup> Implanted perivascular cells regenerate bone indirectly via pleiotropic mechanisms, including for example release of extracellular vesicles (EV)<sup>34</sup> as well as nonvesicular paracrine effectors, such as bone morphogenetic proteins.<sup>36</sup> For example, human perivascular EVs induce osteoprogenitor cell proliferation, migration, and osteogenic differentiation to induce bone repair.<sup>34</sup> In contrast, human perivascular cells inhibit osteoclast formation and prevent bone graft resorption via nonvesicular paracrine mechanisms.<sup>36</sup> Negative regulators of osteoclast differentiation were enriched within perivascular stem cells, including the decoy receptor for RANKL osteoprotegerin (*TNRSF11B*), the Wnt and RANKL inhibitor secreted frizzled-related protein-1 (*SFRP1*), anti-osteoclastic/axonal guidance molecules such as semaphorin 3A (*SEMA3A*), and slit guidance ligand 3 (*SLIT3*). The relative roles of human adventitial cells and pericytes in bone repair were described recently by our group.<sup>37</sup> Here, CD34<sup>+</sup> adventitial cells have a more prominent synthetic role in the formation of bone matrix, whereas CD146<sup>+</sup> pericytes play a supportive role in the induction of blood vessel ingrowth.<sup>37</sup> Other markers that typify adventitial cells have been described in mouse models, including stem cell antigen-1 (*Sca-1*),<sup>38</sup> Gli1,<sup>28</sup> and platelet-derived growth factor receptor (PDGFR)  $\alpha$ .<sup>32</sup> The expression of PDGFR $\alpha$  on most adventitial cells brings to the fore the possible overlap in terminology between fibro-adipoprogenitor cells (FAPs) and adventitial cells. Certainly soft tissue resident FAPs, like adventitial cells, have been described to ossify under appropriate contexts.<sup>39,40</sup> Although adventitial cells have been clearly identified as an osteogenic precursor, the heterogeneity within this cell population has been increasingly documented.

## 3 | ROLES IN PATHOPHYSIOLOGIC PROCESSES

### 3.1 | Cellular heterogeneity within the tunica adventitia of mice

The functional study of subsets of adventitial cells has been possible by the generation of different mouse models that helped track the origin and contribution of these cells during injury and disease as well as tissue homeostasis. Indeed, different groups have identified subsets of adventitial cells involved in fibrosis, calcification, and regeneration. Earlier studies implicated Sca-1- and PDGFR $\beta$ -expressing adventitial cells enriched for sonic hedgehog (Shh) signaling activity as cells with a stem-like identity.<sup>41</sup> For instance, Gli1<sup>+</sup> adventitial cells are myofibroblast progenitors and contribute to fibrosis in different organs.<sup>42,43</sup> Moreover, this same population of adventitial cells expressing Gli1 can migrate to the intima, become osteoblast-like

cells and contribute to vessel calcification during chronic kidney disease (CKD).<sup>28</sup> In the bone marrow  $\text{Gli1}^+$  mesenchymal cells contribute to bone marrow fibrosis and dysregulation of HSCs.<sup>43</sup> In this context,  $\text{Gli1}$  appears to be a pan-marker of fibrotic cells in different organs making it a potential therapeutic target. Nonetheless, the adventitia shows high heterogeneity and other markers have been described.

Understanding of the mechanisms by which perivascular cells contribute during the regeneration process is crucial to develop new strategies to treat diseases such as fibrosis. In specific, the identification of functional subsets is important to either inhibit or promote a given cell fate and improve tissue regeneration. For example, Rafael Kramann's group has recently reported a cell atlas of both human and mouse kidney in which they identified subpopulations of mesenchymal cells including perivascular cells as likely contributors to kidney fibrosis and furthermore described Naked Cuticle Homolog 2 ( $\text{Nkd2}$ ) as a specific myofibroblast target.<sup>44</sup>

$\text{PDGFR}\alpha$  and  $\text{PDGFR}\beta$  play key roles in mesenchymal biology. Both of these receptors are involved in cellular proliferation, migration, and differentiation.<sup>45</sup> Moreover, subsets of cells expressing  $\text{PDGFR}\alpha$ ,  $\text{PDGFR}\beta$ , or both have divergent functions in regeneration. For example,  $\text{PDGFR}\alpha^+\text{PDGFR}\beta^+$  perivascular cells within skeletal muscle have been observed to have fibroadipogenic properties, whereas  $\text{PDGFR}\beta^+\text{PDGFR}\alpha^-$  perivascular cells have regenerative/myogenic features.<sup>32</sup> In skeletal muscle and cardiac tissue,  $\alpha v$  integrins on  $\text{PDGFR}\beta^+$  perivascular cells promote the formation of fibrotic tissue.<sup>24</sup> In adipose tissue,  $\text{PDGFR}\alpha/\text{PDGFR}\beta$  regulate cell differentiation into white or brown adipocytes as well as transition into myofibroblasts.<sup>46,47</sup>

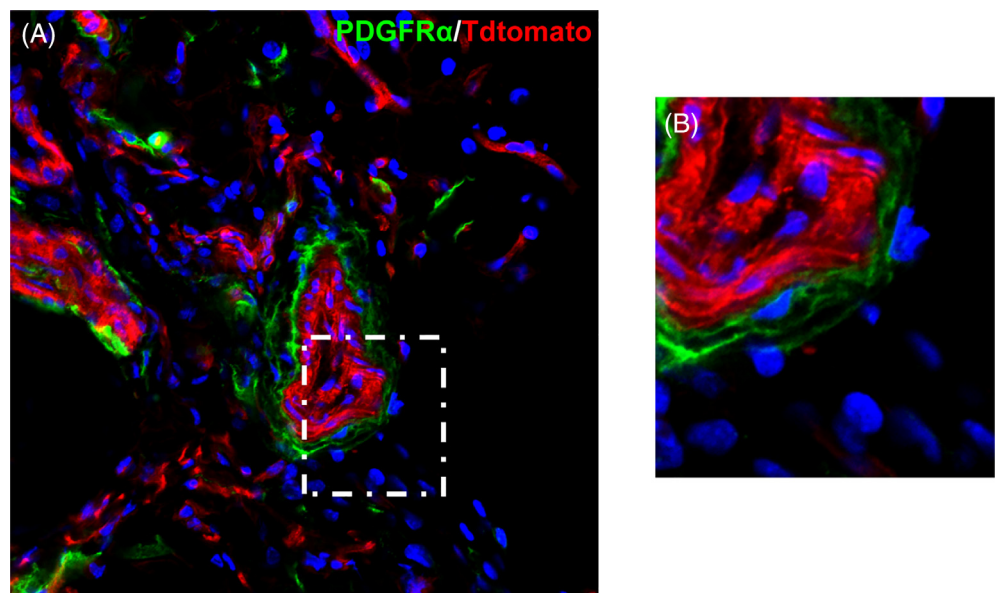
The use of  $\text{PDGFR}\alpha$  reporter activity within mouse white adipose tissue to differentiate subsets of adventitial cells has been recently described<sup>32</sup> (Figure 1).  $\text{PDGFR}\alpha$  reporter cells are located predominantly in the inner layer of the adventitia, while the cell surface marker  $\text{CD34}$  highlights the majority of this layer.<sup>32</sup> Using fluorescence-activated cell sorting (FACS) isolation of adventitial cell subsets,  $\text{PDGFR}\alpha$  and  $\text{CD34}$

coexpressing adventicytes showed greater osteogenic potential than  $\text{PDGFR}\alpha^+$  only or  $\text{CD34}^+\text{PDGFR}\alpha^-$  cells.<sup>32</sup> Indeed  $\text{PDGFR}\alpha^+$  perivascular cells demonstrated more stem cell features than other cell fractions. In addition to higher proliferation rate,  $\text{PDGFR}\alpha^+$  cells repopulated the *tunica adventitia* more effectively than  $\text{PDGFR}\alpha^-$  perivascular cells upon isolation and retransplantation. However, the stem-like identity of  $\text{PDGFR}\alpha$ -expressing adventitial cells was restricted to bone and adipose lineages. For example, long-term lineage tracing failed to reveal any contribution of  $\text{PDGFR}\alpha$ -expressing adventicytes to the smooth muscle medial layer in homeostatic conditions.<sup>32</sup> When implanted with bone graft material,  $\text{PDGFR}\alpha^+$  cells participated in osteoblastogenesis to a greater degree than  $\text{PDGFR}\alpha^-$  perivascular cells. Finally, upon stimulation with  $\text{BMP2}$ , endogenous  $\text{PDGFR}\alpha^+$  reporter cells and their cellular descendants became osteoblasts, adipocytes, and new perivascular cells within new-formed ossicles.<sup>32</sup> These results parallel experimental studies in skeletal muscle where a large portion of  $\text{PDGFR}\alpha$ -expressing cells give rise to dystrophic calcification and ossification during heterotopic bone formation (Table 1).<sup>41</sup>

### 3.2 | Cellular heterogeneity of the human adventitia

Less is known about the mechanism regulating vascular stem cells in the human adventitia, and whether the subsets described in mice have analogous counterparts in the human vasculature. In vitro studies of FACS sorted perivascular cells, transcriptomic analysis, and immunohistochemistry on tissue samples from healthy and diseased individuals can help us understand the mechanisms by which these cells contribute to regeneration and link findings from mouse models to human pathobiology. For example, Kramann et al extended their findings on vascular calcification during mouse CKD by performing  $\text{Gli1}$  immunohistochemistry on human arteries obtained from dialysis-dependent and non-CKD subjects. Expression of  $\text{Gli1}$  in non-CKD patients was mainly found in the

**FIGURE 1**  $\text{PDGFR}\alpha$  marks a population of cells within the *tunica adventitia*. A,  $\text{PDGFR}\alpha^{\text{mT/mG}}$  reporter mice contain green  $\text{PDGFR}\alpha^+$  cells within the *tunica adventitia* in the inguinal fat pad. All other cells are red. Nuclear counterstain appears in blue. B, High magnification of the *tunica adventitia*



adventitial layer, whereas in dialysis-dependent patients Gli1 expression was present in the calcified media and atherosclerotic plaque.<sup>28</sup> This suggests that Gli1 has similar functions in human and mouse arteries, making it a possible therapeutic target in vascular calcification. We have identified cell subsets in the human adventitia expressing CD10 or CD107a, and distinct differentiation potentials.<sup>33,48</sup> Transcriptomic analysis of human adventitial cells also revealed that high aldehyde dehydrogenase (ALDH) activity marks stem cell-like cells.<sup>29</sup> In this section, we will discuss in detail these novel markers of the human adventitia (Table 1).

ALDH activity has been used as a marker of stem cells: hematopoietic and neural stem and progenitor cells exhibit high ALDH activity,<sup>49</sup> also reported in adipose tissue<sup>50</sup> and myogenic progenitors.<sup>51</sup> On the other hand, high ALDH activity has been linked, in various cancers,<sup>52</sup> to stem cell features such as tumor initiation, clonogenic growth, self-renewal, and drug resistance.<sup>53,54</sup> Hardy et al analyzed gene expression in single human pericytes and adventitial cells further separated according to ALDH activity and revealed the existence of a developmental hierarchy of human perivascular cells, ranging from ALDH high adventicytes (most primitive) to ALDH low pericytes (least primitive). Adventitial cells show a distribution of cells ranging from low to high ALDH activity, whereas pericytes exhibit mostly low ALDH activity, suggesting that adventitial cells contain more stem cell-like cells than pericytes do. Therefore, the *tunica adventitia* and more specifically adventitial cells with high ALDH activity may contain cells with stem cell properties.<sup>29</sup> The stem cell properties of this subset of adventitial cells may be related to the involvement of ALDH isoforms in the retinoic acid (RA) pathway. For instance, the ALDH1 family of enzymes regulates cell proliferation and differentiation by converting oxidase retinaldehyde (retinal) to RA, which subsequently interacts with nuclear receptors to promote gene transcription.<sup>28,55</sup> Lastly, unpublished data from our group indicate the existence of a specific isoform from the ALDH1 family expressed in adventitial cells (Gomez-Salazar et al, in preparation).

CD10, also known as neprilysin, or membrane metalloendopeptidase, is a zinc-dependent metalloendoprotease involved in peptide signaling. CD10 regulates the extracellular concentration of various peptides, changing the availability for receptor binding and therefore regulating biological processes.<sup>48</sup> Expression of CD10 plays key roles in the regulation of stem cells by cleaving peptides which are then either activated or inhibited to continue or stop the signaling cascade.<sup>56</sup> For example, CD10 regulates cell migration and angiogenesis through Akt, Rho, and FGF signaling.<sup>57,58</sup> CD10 is also highly expressed in leukemia and in solid childhood tumors including nephroblastoma and neuroblastoma,<sup>59</sup> and is used as a marker of good prognosis in certain types of leukemia.<sup>60</sup> On the other hand,

CD10 expression also identifies normal stem cells in different tissues including hematopoietic (lymphoid) progenitors, as well as other organ systems.<sup>61,62</sup> In the context of vascular biology, our group identified a novel CD10<sup>+</sup> adventitial progenitor cell type with higher proliferation rate and osteogenic differentiation potential compared with the negative population, suggesting pathological functions during vessel remodeling.<sup>48</sup> Ding et al showed that expression of CD10 by adventitial cells is regulated through SHH/Gli1, which is interesting since Gli1 is involved in vessel calcification. CD10<sup>+</sup> adventitial cells express genes related to stem cell potential, such as SRY-box transcription factor 2 (SOX2) and NANOG, as well as the cell proliferation-related gene cell cycle G1/S-specific cyclin D2 (CCND2). Moreover, CD10<sup>+</sup> adventitial cells strongly express neural epidermal growth factor-like 1 (NELL1), which is a promoter of bone development. Whether bone regeneration or vascular calcification directly involve CD10, or whether this is a mere marker of a functional cell subset within the adventitia, is not known yet.

CD107a, also known as lysosome-associated membrane protein-1 (LAMP1), is a type 1 membrane protein highly expressed in lysosomes and other intracellular vesicles.<sup>63</sup> While CD107a is ubiquitously expressed intracellularly, only a fraction of mammalian cells display detectable surface CD107a. Our group recently identified surface CD107a as a marker to segregate functionally relevant cells within the human adventitial cell niche.<sup>33</sup> CD107a immunoreactivity is found most frequently within the outermost layers of blood vessels, and more common in the outer *tunica adventitia*. FACS-derived CD107a<sup>low</sup> and CD107a<sup>high</sup> stromal cells from human white adipose tissue have opposite differentiation potentials. The CD107a<sup>low</sup> stromal component contains a precursor cell population with high osteoblastogenic potential, while CD107a<sup>high</sup> cells represent an adipocyte precursor cell. Transcriptomic analysis demonstrates that genes associated with adipogenic differentiation, such as *FABP4* (fatty acid binding protein 4), *LPL* (lipoprotein lipase), *PPARGC1A* (PPARG coactivator 1  $\alpha$ ), and *CEBPA* (CCAAT enhancer binding protein  $\alpha$ ), are highly expressed among CD107a<sup>high</sup> stromal cells. Conversely, negative regulators of adipogenesis, such as *KLF2* (Krüppel-like Factor 2), *KLF3*, *SIRT1* (sirtuin 1), and *DDIT3* (DNA damage inducible transcript 3), are increased among CD107a<sup>low</sup> stromal cells. In addition, CD107a<sup>low</sup> stromal cells are enriched for signaling pathways associated with bone formation and cellular respiration and metabolism, including Wnt/ $\beta$ -catenin signaling, oxidative phosphorylation, and glutathione metabolism. CD107a<sup>low</sup> cells also drive higher osteogenic differentiation in vivo. Xenotransplantation confirmed significant quantitative differences in bone generation among CD107a cellular subsets. Briefly, an accumulation of new bone at 8 weeks was observed after intramuscular implantation in NOD-SCID mice of CD107a<sup>low</sup> rather than CD107a<sup>high</sup> sorted

Markers	Organisms	Function	Reference
ALDH <sup>high</sup>	Human	Osteogenic and adipogenic potential (predicted)	Hardy et al <sup>29</sup>
PDGFR $\alpha$ <sup>+</sup>	Mice	Osteogenic and adipogenic potential	Wang et al <sup>32</sup>
CD107a <sup>high</sup>	Human	Adipogenic potential	Xu et al <sup>33</sup>
CD10 <sup>+</sup>	Human	Osteogenic potential	Ding et al <sup>48</sup>

**TABLE 1** Phenotypes and functionalities of arterial adventitial cells in man and mice

cells. Human CD107a<sup>low</sup> cells also increased posterolateral lumbar spine fusion in athymic rats. Analyses performed after 8 weeks demonstrated 62.5% spine fusion among CD107a<sup>low</sup> cell-treated animals, whereas CD107a<sup>high</sup> cell-transplanted animals only showed 37.5% fusion. In summary, these studies pointed to CD107a<sup>low</sup> mesenchymal cells as a cell subset with higher osteogenic potential. Interestingly, and as expected from functional differences, zones of expression of CD10 and CD107a within the *tunica adventitia* of vessels are distinct (Figure 2).

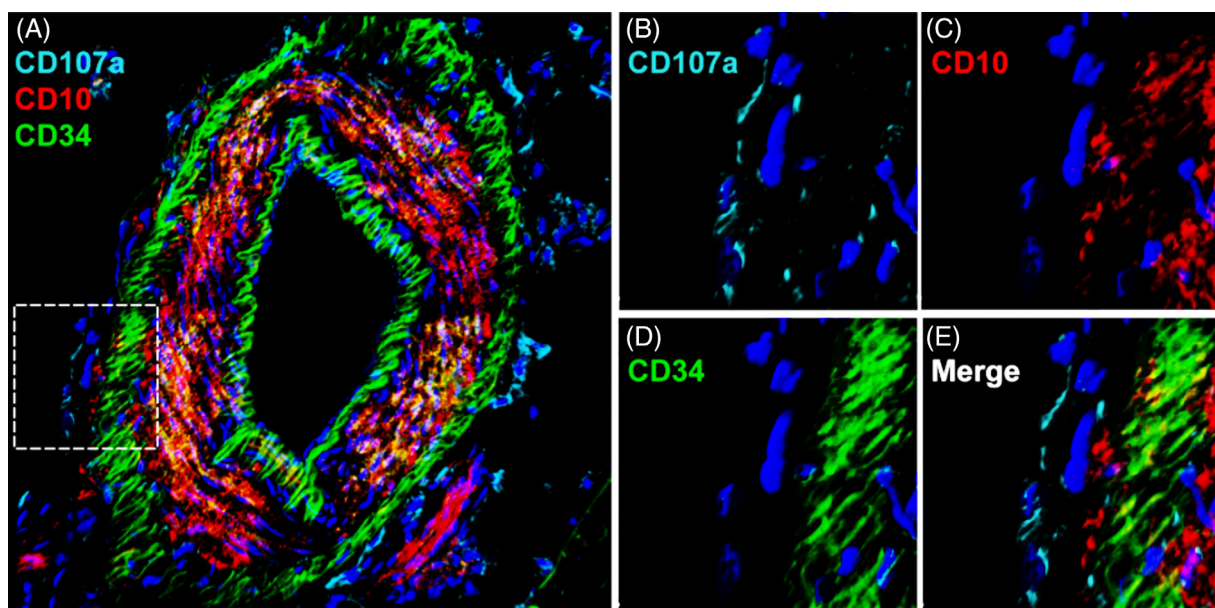
#### 4 | CLINICAL APPLICATION AND PERSPECTIVES

Despite the high number of preclinical studies showing positive results with the use of mesenchymal progenitor cells, their use in the clinical setting is limited.<sup>64</sup> Among the factors affecting efficiency is the use of total cell preparations containing subsets that may hinder the efficacy of regeneration, resulting in inconsistent clinical outcomes. Moreover, clonal selection within total cell preparations may further reduce numbers of highly regenerative progenitor cells. Our group specially focused on elucidating functional heterogeneity of perivascular cells that may contribute to standardizing cell preparations and improving clinical outcomes. For instance, we have shown that CD10<sup>+</sup> cell preparations have increased osteogenic potential, which will likely enhance regeneration in skeletal injuries. Tailoring of cell therapies for specific pathologies may represent a step forward in realizing the potential of multipotent progenitor cells for tissue engineering.<sup>7</sup>

Not discussed here, the vehicle or scaffold for cell deployment is also vitally important for efforts in skeletal tissue regeneration. Progenitor cells are highly influenced by their microenvironment, and the physical and molecular characteristics of a given scaffold will result in shifts in cell phenotype and functional outcomes in terms of tissue formed. One such example using human perivascular cells was recently reported, where tunable supramolecular hydrogels along with different stiffnesses exert changes in pericyte differentiation toward osteogenic and chondrogenic lineages.<sup>65</sup>

#### 5 | CONCLUSION

Despite its relatively unremarkable histologic appearance, the *tunica adventitia* houses a wealth of cell types—some of which have mesenchymal progenitor cell attributes. This brief review covered only some of the established and emerging markers in mouse and human tissues that resolve functionally relevant subsets of perivascular cells. In addition to harboring progenitor cells, the adventitial layer is a major site of accumulation of immune cells including macrophages, lymphocytes, mast cells, and dendritic cells that carry out important surveillance and innate immune functions in response to foreign antigens and play a role in vascular pathologies including atherosclerosis and tissue fibrosis.<sup>66</sup> Whether a specific subset of mesenchymal progenitor cells in this perivascular niche is involved in immune regulation and subsequent tissue remodeling is yet to be investigated. A critical point is that many markers used to purify cells within the *tunica adventitia* are also present in minor degrees in other cellular locations, such as the perineural tissues or fascia of white adipose tissue. The fascia is a



**FIGURE 2** CD10 and CD107a mark distinct subpopulations within the *tunica adventitia*. A-E, Immunofluorescent staining for CD107a, CD10, and CD34 in an artery within human subcutaneous white adipose tissue. A, Whole vessel in cross-section showing CD34 expression in the endothelial and adventitial layers. CD10 and CD107a expression are seen on different subsets in the inner and outer adventitia. B-E, High magnification of the *tunica adventitia*

framework of connective tissue that envelops and separates organs and tissues.<sup>67</sup> In adipose tissue, the fascia contains preadipocytes with high differentiation potential.<sup>68</sup> Cells in the fascia express markers shared with perivascular cells such as CD34 and CD44.<sup>69</sup> In a similar manner, perineural cells express markers also found in mesenchymal cells such as vimentin, CD34, and  $\alpha$ -SMA.<sup>70,71</sup> Moreover, during development in zebrafish and mouse, Schwann cell precursors give rise to mesenchymal progenitors that subsequently differentiate into chondrocytes and osteocytes, describing a common developmental origin that may explain why they share similar expression patterns with adventitial cells.<sup>72</sup> All this exemplifies the complexity of purifying and studying perivascular progenitor cells. Until we have more specific markers for adventitial cells, purification of perivascular progenitors will be prone to contain a fraction of other cell types. Importantly, the inherent regenerative potential of specific subsets of adventitial cells will further improve efficiency and consistency when used in bioengineering approaches.

This review focused on adipose tissue perivascular cells, but similar cells within the bone marrow are also well characterized as multipotent progenitors, termed CXC chemokine ligand (CXCL) 12-abundant reticular (CAR) cells or leptin-receptor-positive (LepR<sup>+</sup>) stromal cells. LepR<sup>+</sup> cells are the major source of bone and adipocytes in adult bone marrow.<sup>73</sup> Short-term ablation of CAR cells in vivo impairs osteogenesis from marrow cells.<sup>74</sup> Furthermore, CAR cells and LepR<sup>+</sup> stromal cells have been implicated in maintaining the quiescent HSC pool and appear to be a key component of HSC niches.<sup>75,76</sup> Mechanistically, Foxc1, expressed in CAR cells, is essential for HSC maintenance and promotes CAR cell development by upregulating CXCL12 and stem cell factor expression.<sup>77</sup> In addition, the transcription factor early B-cell factor 3 (Ebf3) is preferentially expressed in CAR/LepR<sup>+</sup> cells, required to create HSC niches and maintain spaces for HSCs.<sup>78</sup> In contrast to adipose tissue as discussed above, bone marrow perivascular cells are primarily housed within microvessels and have a perivascular position consistent with pericytes. To our knowledge, adventitial cells have not been isolated or characterized from skeletal sources.

Several unanswered questions regarding these recent findings are most notable. For example, distribution of novel markers such as CD10 and CD107a suggests a microarchitectural spatial organization of the *tunica adventitia* within fat tissue that had been previously under-recognized. Yet, the broader spatial relations between adventitial cells, and whether these are conserved across organ systems remain unknown. Most obviously, the hunt for a definitive stem cell within the *tunica adventitia*—one with self-renewal potential—is a matter of considerable interest. Certainly, identification of more primitive/progenitor cell types within vessel walls has broad implications for vascular biology, but also usefulness in the field of tissue engineering and regenerative medicine.

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#### CONFLICT OF INTEREST

A.W.J. is a paid consultant for Novadip LLC and Lifesprout LLC. This arrangement has been reviewed and approved by the JHU in accordance with its conflict of interest policies. A.W.J. receives funding for unrelated research from MTF Biologics and Novadip, and is on the editorial board of American Journal of Pathology and Bone Research. A.W.J. is the inventor of methods to purify CD107a progenitor cells held by the Johns Hopkins University. B.P. is the inventor of perivascular stem cell related patents held by the UC Regents and is on the editorial board of Stem Cells. The other authors declared no potential conflicts of interest.

#### AUTHOR CONTRIBUTIONS

J.X., Y.W., M.G., A.W.J.: conceptualization; G.H., S.N., Z.L., W.H., L.D.: visualization; J.X., Y.W., M.G., A.W.J.: writing—original draft; B.P., A.W.J.: writing—review & editing.


#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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