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CONCISE REVIEW

Defining Vascular Stem Cells

Ching-Shwun Lin and Tom F. Lue

Mesenchymal stem cells (MSCs) exist in most adult tissues and have been located near or within blood vessels. Although "perivascular" has been commonly used to describe such locations, increasing evidence points at the vessel wall as the exact location. Thus, "vascular stem cells (VSCs)" is recommended as a more accurate term for MSCs. Furthermore, 2 cell populations, namely pericytes and adventitial progenitor cells (APCs), are the likely VSCs. The pericyte evidence relies on the so-called pericyte-specific markers, but none of these markers is pericyte specific. In addition, pericytes appear to be too functionally diverse and sophisticated to have a large differentiation capacity. On the other hand, APCs are more naïve functionally and, therefore, more akin to being VSCs. In vitro, these cells spontaneously differentiate into pericytes, and can be induced to differentiate into vascular cells (endothelial and smooth muscle cells) and mesenchymal cells (eg, bone, cartilage, and fat). In vivo, indirect evidence also points to their ability to differentiate into mesenchymal cells of their native tissue (eg, fat). Moreover, they possess a large paracrine capacity and, therefore, can help maintain tissue homeostasis by encouraging the replication and differentiation of mesenchymal cells locally. These proposed in vivo functions are areas of interest for future research on VSCs.

Introduction

A recent study on vascular stem cells (VSCs) [1] has generated great interests in the media, many of which hailed the discovery of such cells. However, "vascular stem cells" as a biomedical term first appeared more than 10 years ago, and their existence in humans and experimental animals was demonstrated another decade earlier. Granted, these cells remain poorly understood and not well known even in the stem cell research field. There is, however, accumulating evidence that they are the de facto mesenchymal stem cells (MSCs) which have already entered the clinical trial arena. Thus, this review is intended to provide a definition for VSCs, discuss the evidence for the VSC-MSC connection, and propose a working model for VSC's role in tissue development and regeneration.

What Are "Vascular Stem Cells"?

Exhaustive search of the literature found the term "vascular stem cell" in some 30 articles. In most cases, it was, or at least seemed to have been, used casually or accidentally to denote any stem or stem-like cell that has a connection to the vascular system, be it blood or blood vessels. Thus, vastly different usages for the term "vascular stem cells" can be seen scattered in the literature. However, amid all these confusions, 2 articles each provided a definition. The first, which is concerned with angiogenesis and lymphangiogenesis, defined VSCs as "stem cells that can differentiate into endothelial or mural cells in the blood vessel wall" [2]. The second, which is truly dedicated to the discussion of stem cells in the vasculature, defined VSC as "a putative self-renewing multipotent stem cell that gives rise to vascular lineages" [3]. While the first definition seems more definitive (confinement to blood vessel wall), the term "mural cells" would require a separate definition. On the other hand, the second definition is vague in the sense that "vascular lineages" may not be confined to blood vessel walls. More importantly, both of these 2 definitions are missing the most critical element in the definition of VSCs; that is, they do not specify where these cells come from. Without this element, any stem cells, including those that have no direct vascular connection (eg, embryonic stem cells and epithelial stem cells), can be VSCs, as long as they can, or can be induced to, differentiate into vessel wall cells. Thus, in order to eliminate such ambiguities, "VSCs" is defined in this article as "cells that reside within the blood vessel wall and can differentiate into all of the cell types that constitute a functional blood vessel." Specifically, the cell types that VSCs can differentiate into include endothelial cells and pericytes in the capillary, and additionally, smooth muscle and adventitial cells in larger vessels.

Vascular or Perivascular

MSCs were first identified in the bone marrow but are now known to exist in most, if not all, adult tissues. Their function is believed to serve as a cell reservoir for the normal

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turnover and maintenance of adult mesenchymal tissues [4]. However, due to the lack of definitive cellular markers, their location within each tissue has not been precisely determined. Increasingly though, they are believed to reside near or within blood vessels, and often such locations are called "perivascular." While such a term is as "safe" as it is being ambiguous (near or within?), accumulating and improving histological data have, by and large, eliminated "near blood vessels" as a choice location. In particular, MSCs of adipose tissue [adipose-derived stem cells (ADSCs)] are routinely isolated from the stromal vascular fraction and have been consistently localized within blood vessels [5,6]. Specifically, 2 intra-vessel wall compartments, namely the adventitia and the sub-endothelium, have emerged as the most plausible locations for MSCs. Furthermore, with regard to the sub-endothelial location, many studies have concluded that pericytes are the de facto MSCs [7–9]. Thus, the term "perivascular" is becoming increasingly less able to accurately reflect the vascular nature of MSCs.

Endothelial Progenitor Cells as Vascular Stem Cells

The term "vascular stem cells" first appeared in the 2001 December issue of "Journal of Hematotherapy & Stem Cell Research," which was renamed "Stem Cells and Development" in 2004. In this issue, a section named "Vascular Stem Cells and Angiogenesis" contains a single paper titled "In Vivo Absence of Synergism Between Fibroblast Growth Factor-2 and Vascular Endothelial Growth Factor" [10]. This paper, however, is concerned with angiogenesis, not VSCs, and it mentions no VSCs.

In the next issue (February 2002) of the same journal, an editorial titled "Vascular Stem Cell Transplantation: The Next Phase of Hematotherapy?" begins with "Vascular stem or progenitor cells reside in the bone marrow where they exist as a small subset of CD34+ cells" [11]. It also states: "We owe much of our present knowledge of vascular stem cells to Dr. Jeffrey M. Isner." Thus, clearly, this journal and its editor used the term "vascular stem cells" to mean "endothelial progenitor cells (EPCs)," as Dr. Isner has been credited with the discovery of EPCs. In addition, in the next issue (April 2002) of the same journal, a section named "State-of-the-Art Reviews on Vascular Stem Cells and Angiogenesis" indeed contains a paper coauthored by Dr. Isner and that is titled "Endothelial Progenitor Cells for Vascular Regeneration" [12]. Thus, there is no doubt that in 2001 and 2002, this journal used the term "vascular stem cells" synonymously with "endothelial progenitor cells." Of note is that none of the papers contained within these issues of this particular journal mentions "vascular stem cells" or "VSCs" in their texts, and since 2002, the EPC-as-VSC stance could no longer be found in this journal.

However, equating VSC with EPC is not unique to the journal just mentioned. In 2003, a paper titled "Endothelial progenitor cells: the promise of vascular stem cells for plastic surgery" appeared in Plastic and Reconstructive Surgery [13]. In 2011, another review article titled "Vascular stem cells and ischaemic retinopathies" [14] also, by and large, endorses the EPC-as-VSC concept. Similarly, and perhaps more dramatically, in 2012, an entire issue of Experimental Diabetes Research is dedicated to "Vascular stem and progenitor cells in diabetic complications" [15]. While it contains a total of 10 original research and review articles that are concerned exclusively with EPCs, none of these articles contain "vascular stem cells" or "VSCs" in their own texts. On the other hand, a separate paper with a similar title (Vascular stem cells in diabetic complications) talks about the contribution of EPCs and VSCs to the pathogenesis of diabetic complications [16]. However, in this article, "VSCs" is used to denote any cells (eg, embryonic stem cells) that can be induced in vitro to become vascular cells [endothelial cells and vascular smooth muscle cells (SMCs)]. Thus, the so-called VSCs are not vascular *per se*, and should not be confused with the VSCs that are truly vascular and are the subject of the present article.

While not a focus of this review, controversies surrounding the identity of EPCs should be briefly mentioned. In particular, it has been shown that EPCs might be monocytes contaminated with platelet microparticles [17], or they were injured or senescent endothelial cells that slough from the vessel wall into the bloodstream [18]. More importantly, 2 experts in the EPC field have proposed that the term EPC be retired, because mounting evidence indicates that different EPC subsets are, in fact, various lineages of hematopoietic cells [19].

Multipotent Vessel Wall Cells as Vascular Stem Cells

While EPCs are defined as originating from bone marrow and circulating in the blood, "true" VSCs (as defined earlier, under "What is vascular stem cells") are expected to reside within the blood vessel wall. At this location (vessel wall), 3 distinct cell types exist: endothelial cells and pericytes in capillaries, and additionally, SMCs in larger vessels. In addition, a mixed population of cells (macrophages, dendritic cells, fibroblasts, and progenitor cells) resides in the adventitia [20,21]. The possibilities for these vessel wall cells to be stem or progenitor cells have been reviewed, for example, in these recent articles [3,20–29]. In particular, pericytes and adventitial progenitor cells (APCs) have emerged as the most plausible VSCs and, thus, will be the focus of the present review.

Pericytes as Vascular Stem Cells

The term "pericyte" was introduced in 1923 to denote a type of cells whose branching cytoplasmic processes partially encircle endothelial cells from the abluminal side of microvessels [30]. However, nearly 90 years later, there is still no universally accepted definition for pericytes [31–33]. Nowadays, an in vivo identification of pericytes is done almost exclusively by perivascular immunolocalization for "pericyte-specific" markers [31–33]. In addition, validation of cultured pericytes also relies mostly on immunocytochemistry with pericyte-specific markers [31–33]. Therefore, the importance of these so-called pericyte-specific markers cannot be overemphasized. However, many studies have shown that some of these markers fall short of being pericyte specific, and virtually all review articles on this subject have mentioned the nonspecific nature of these markers [31–33]. As such, erroneous interpretations might have been made in some studies,

particularly those that relied on a single pericyte marker for the isolation or identification of candidate pericytes. With these caveats in mind, studies that support pericytes as potential VSCs are discussed next. However, before proceeding further, it should be mentioned that pericytes are increasingly considered as the de facto MSCs, and this is partly based on demonstrations of the pericytes' ability to differentiate into osteoblasts, chondrocytes, and adipocytes. As such, discussions next will mostly center on this tri-lineage differentiation.

The first study implicating the osteogenic potential of pericytes was published in 1990 [34]. After isolation from bovine retina and characterization by growth and morphological properties, pericytes were found to spontaneously form nodules in culture, and which were further identified to contain alkaline phosphatase and hydroxyapatite crystals. In 1998, this research team provided further evidence for pericytes being osteogenic and chondrogenic. Specifically, they inoculated bovine retina pericytes into diffusion chambers, and intraperitoneally implanted these pericytes-containing chambers into athymic mice. Eight weeks later, the chambers were found to contain a matrix of bone, cartilage, and fibrous tissue [35]. In 2004, they further reported that, when cultured in a chondrogenic medium, bovine retina pericytes formed pellets of cells that were embedded in an extracellular matrix which was rich in sulfated proteoglycans and type II collagen [36]. Additionally, when cultured in an adipogenic medium, pericytes expressed mRNA for adipocyte-specific transcription factor PPAR-γ2 and incorporated lipid droplets. In chamber assays, both chondrocytes and adipocytes were also identified. Thus, pericytes appear to fulfill the tri-lineage differentiation criteria for being MSCs.

Possible osteogenic differentiation of pericytes was also independently demonstrated in two 1992 studies. In one, monastral blue was used to label pericytes and endothelial cells in the postcapillary venules of rat femur; 3 to 6 days later, monastral blue was observed in some osteoblasts [37]. In the other, pericytes isolated from bovine brain and cultured in standard medium were found to spontaneously synthesize alkaline phosphatase, form mineralized colonies, and express osteocalcin [38]. The authors thus concluded that pericytes are possible osteoblast progenitor cells. However, in this study, validation of pericyte identity was done by immunofluorescence for smooth muscle actin (SMA), which is obviously not a pericyte-specific marker. In fact, a recent study even showed that SMA is a negative marker for pericytes [39], with additional reference to another study that stated: "few freshly isolated pericytes express this antigen, but with time in culture, almost 100% of cells express α-SMA" [40].

In a 1993 study, calcified human carotid atherosclerotic lesions were found to contain bone morphogenic protein (BMP)-2a mRNA, and cultured bovine and human aortic medial cells formed nodules that stained positive for SMA and 3G5 (both were considered pericyte markers) [41]. Thus, the authors concluded that pericyte-like cells were capable of osteoblastic differentiation. In subsequent studies, the pericyte-like cells were called calcifying vascular cells (CVC) [42] and found capable of chondrogenic, leiomyogenic, stromogenic, but not adipogenic differentiation [43]. In a subsequent review article and an editorial by this research team, the term "vascular stem cells" emerged, albeit without definition [44,45]. The extensive reference to pericytes and MSCs in the review article, nevertheless, hints at the authors' intent to equate CVC with VSC, pericytes, and MSCs [44]. In their more recent reviews, this intent is further illustrated [46,47].

In a 2004 study, vascular smooth muscle cells (VSMCs) and pericytes were reported to be the progenitor cells of Leydig cells [48]. In the experiment, Leydig cells were destroyed by an intraperitoneal injection of ethane dimethane sulphonate in adult rats. Spontaneous regeneration of Leydig cells was then traced to the testicular vasculature by immunostaining for nestin. The nestin-positive cells were then further identified as pericytes or VSMCs by immunostaining for SMA. Thus, the "pericytes as Leydig cell progenitor" theory depends on the reliability of nestin and SMA as markers for Leydig cells and pericytes, respectively. In addition, since VSMCs were also shown to be possible Leydig cell progenitors, the "pericytes as Leydig cell progenitor" theory gets diluted in terms of believability. Furthermore, among recent review articles on the subject of Leydig cell progenitors [49–51], only one has discussed the pericyte theory, and it has further raised the question of whether the regenerated nestin-expressing cells are the same as Leydig cells that developed naturally during the perpubertal period [50].

In a 2008 study, MSCs isolated from various human tissues were compared with pericytes isolated from human retina, and the results showed similarities in marker expression and gene expression profile [52]. However, it should be noted that the retina pericytes were isolated by immune selection for CD146, which is also expressed in nonpericytes, particularly the endothelial cells. Later in the same year, a very similar but independent study also showed similarities between MSCs from various human tissues and pericytes [53]. This time, the pericytes were selected not only for CD146 expression but also for CD34 nonexpression, thus excluding endothelial cells. However, histological images of the cross-sectional view of CD34/CD146 double-stained larger vessels (ie, arterioles) indicated the confinement of CD34 stain inside the CD146-stained ring (Ref. 53's Fig. 1B, C). Such a staining pattern is in disagreement with other studies, in which CD34 stain on perpendicularly cut arterioles produced 2 discrete rings, and these CD34-stained rings sandwiched the CD146-stained ring [54–57]. Particularly, one of these disagreeing studies [57] was recently published from the same lab that produced the study in Ref. [53], and it clearly showed the sandwiching of CD146 stain by CD34 stains. Thus, the conclusion made in Ref. [53] that MSCs might be pericytes was based partly on histological data which may require further clarification.

Similar to the study by Crisan et al. [53], 2 other studies also stated that MSCs isolated from adipose tissue (ADSCs) expressed pericyte markers [58,59]. However, the study by Amos et al. [58,59] provided no histological data on the expression of these markers by ADSCs, and the study by Traktuev et al. [58,59] provided histological data showing ADSCs co-expressing CD34 and pericyte markers. This latter study, thus, disagrees with Crisan et al. [53], which specifically emphasized no such co-expression. Furthermore, several recent studies have shown that CD34 and pericyte

markers are mutually exclusively expressed in the adipose tissue [55–57,60]. Thus, the pericyte-as-ADSC theory will require stronger evidence to be proved.

Adventitial Progenitor Cells as Vascular Stem Cells

The adventitia is the outermost layer of arteries and veins. Unlike the media and intima, which are synonymic with smooth muscle and endothelium, respectively, the adventitia does not have a defined synonymic cell type. However, when an artery's cross-section is stained for CD34, 2 concentric rings of cells corresponding to the intima and adventitia are revealed [54,55,61]. The inner ring is thin, compact, and complete, and is undoubtedly the endothelium that also stains positive for CD31 [54,55,61]. The outer ring, which is outside of the media and thus belongs to the adventitia, is irregular, loose, incomplete, and distinctively CD31− [54,55,61]. This population of CD34+CD31− adventitial cells is now increasingly believed to be VSCs, as discussed next.

In 2004, Hu et al. [62] reported the identification of cells that stained positive for cell markers Sca1, c-kit, CD34, and Flk1 in the adventitia of mouse aorta. In culture, Sca1-expressing adventitial cells differentiated into SMCs in response to PDGF-BB stimulation. Furthermore, when transferred to the adventitial side of vein grafts in ApoE-deficient mice, Sca1-expressing adventitial cells were found in atherosclerotic lesions of the intima. The authors thus concluded that a population of vascular progenitor cells in the adventitia could differentiate into SMCs which contribute to atherosclerosis. In 2007, this team of researchers published a related study in which human, instead of mouse, arteries were examined [63]. Specifically, immunofluorescence identified variable expression of CD34, VEGFR2, Sca1, and c-kit in the adventitia of normal internal mammary arteries and atherosclerotic vessels. Thus, potential stem cells were identified in the adventitia of both human and mouse arteries.

The Sca1 expression mentioned earlier in the adventitia has been independently confirmed in mouse aorta and mesenteric and femoral arteries [64]. More importantly, Sca1+ cells immunoselected from the aortic adventitia were found capable of smooth muscle and endothelial differentiation. Specifically, when cultured in standard medium (DMEM+10% serum), 30%–50% of the Sca1-selected cells lost expression of Sca1 but gained expression of SMC markers (SMA, SM22α, calponin, and SM-MHC). In addition, when the Sca1-selected cells were treated with VEGF, PECAM1+ endothelial cell clusters appeared in elongated cords that often colocalized with SMA+ cells. Moreover, when the Sca1-selected cells were treated with BMP2, colonies formed that stained positive by alizarin red, suggesting osteogenic differentiation. These findings thus reinforced the existence of APCs.

In 2004, Yamashima et al. [65] also reported the identification of adventitial cells that were capable of neuronal differentiation. Specifically, when 5-bromo-2′-deoxyuridine (BrdU) was used to label proliferating cells in ischemic adult monkey brain, 1%–3% of such BrdU+ cells in the subgranular zone were also stained positive for neuronal markers TUC4, beta-III tubulin, and NeuN. In addition, these neuronal progenitor cells were frequently seen in the vicinity of proliferating blood vessels, and electron microscopy indicated that most of the neuronal progenitor cells were pericytes of capillaries and/or adventitial cells of arterioles. Thus, the authors concluded that both pericytes and adventitial cells were capable of neuronal differentiation.

As mentioned earlier, staining of arterial cross-sections revealed 2 concentric CD34+ rings, the outer of which is CD31− and exists in the adventitia. One of the 3 studies that reported this finding was conducted with human internal thoracic artery [61]. When segments of this artery were examined in arterial ring assays, the adventitial CD34+CD31− cells were identified as forming capillary sprouts and expressing markers for angiogenically activated endothelial cells, such as PECAM1, and also for mature endothelial cells, such as VE-cadherin or occludin. This population of CD34+CD31− adventitial cells was also said to exist in all of the human organs studied, including urinary bladder, testis, prostate, kidney, lung, heart, liver, and brain, although only the prostate staining data were shown. The authors thus proposed the term "vasculogenic zone" to denote the CD34+CD31− ring in the adventitia of human blood vessels, and suggested that the CD34+CD31− cells might be progenitor cells for postnatal vasculogenesis. However, in a more recent study by the same research team (with a different first author) and on the same blood vessel (human internal thoracic artery), MSC-like cells residing in the same vasculogenic zone were shown to be CD44+ but CD34− [66]. In the Conclusion section of this study, the authors stated: "… human vessels harbor not only EPCs but also other types of stem cells. …" Thus, it is apparent that the authors considered their previously identified CD34+ adventitial cells as EPCs, while they considered the CD44+CD34− adventitial cells as non-EPC MSCs. While this hypothesis requires further examination, it is noted that the authors offered no rationale for using CD44 as a marker; and, although CD44 has been shown to be highly expressed in cultured MSCs, a recent study provided strong evidence that native MSCs in bone marrow lack CD44 expression [67]. Thus, equating the CD44+CD34− adventitial cells with MSCs will require stronger evidence.

CD34 expression has also been detected by immunohistochemistry in the adventitia of human femoral artery and thoracic aorta [68]. In addition, cells cultured from the aorta assumed a fibroblastic morphology and expressed MSC-associated markers CD44, CD90, and CD105. When treated with VEGF, these cells acquired endothelial characteristics such as capillary-like tube formation and von Willebrand factor expression. Thus, the aorta appeared to harbor a population of EPCs. However, whether these cells were derived from the CD34+ adventitial cells was not demonstrated in this study.

In a 2010 study, Campagnolo et al. [69] reported the identification of CD34+CD31− cells in the adventitia of human vena saphena. A few of these cells were also described as staining positive for pericyte markers NG2 and PDGFRβ. However, the displayed histological images actually show neighboring yet mutually exclusive expression between CD34 and NG2, and between CD34 and PDGFRβ. Moreover, although immunocytochemical analysis showed

positivity for NG2 and PDGFRβ in immunoselected CD34+CD31− cells from this blood vessel, no co-expression with CD34 was determined. Regardless, these cells were tested positive for osteogenic, adipogenic, neurogenic, and myogenic differentiation but negative for chondrogenic differentiation. Interestingly, although these cells were described as negative for endothelial differentiation, the actual data of capillary-like tube formation do suggest such differentiation. Furthermore, when injected into the induced ischemic limbs of mice, these cells improved neovascularization and blood flow recovery, thus suggesting their angiogenic potential. In any event, although these cells were interpreted as MSCs/pericytes, they should be considered MSCs/adventitial cells because of their adventitial localization, CD34 expression, and lack of pericyte marker expression.

As mentioned earlier, 3 studies have reported that MSCs in the adipose tissue (ADSCs) are possibly pericytes [53,58,59]. However, Amos et al. [58] provided in vitro but not in vivo data, while Crisan et al. [53] and Traktuev et al. [59] disagreed on whether ADSCs express CD34 or not. Specifically, while Crisan et al. [53] used anti-CD34 antibody to select against endothelial cells (thereby enriching the CD34− pericyte fraction), Traktuev et al. [59] used CD34 as a marker for pericytes. Based on the consensus view that native ADSCs are CD34+ [70], the CD34− pericytes reported by Crisan et al. [53] cannot be ADSCs. On the other hand, while CD34 was correctly used as an ADSC marker by Traktuev et al. [59], the data showing the longitudinal view of blood vessels do not permit the visualization of the layer in which CD34 stains were located. More importantly, the co-expression of CD34 and pericyte markers was assessed by flow cytometry on cultured, not native, ADSCs. Thus, such co-expression is possibly a consequence of cell culture, as demonstrated in 4 recent studies [57,60,71,72].

The first study dedicated to the histological analysis of ADSCs in the adipose tissue was published in 2008 [55]. In this study, human adipose tissue was stained for CD34 and several other markers, including CD31, SMA, and CD140b. Although CD34 is considered an ADSC marker, it is also expressed in endothelial cells; therefore, CD31 co-staining was used to distinguish between ADSCs (CD34+CD31−) and endothelial cells (CD34+CD31+). In addition, SMA co-staining with CD34 and CD31 was used to visualize the adventitia-media and endothelium-media boundaries, respectively. After these co-stainings, along with co-staining for several other potential stem cell markers (Stro1, Oct4, SSEA1, and telomerase), the cross-sectional views of blood vessels of various sizes (arteries, arterioles, and capillaries) were compiled and analyzed. The results showed CD34 expression in both the endothelium and the adventitia of arteries and arterioles, with SMA expression sandwiched in between. With regard to capillaries, despite their lack of layered structure-like arteries, the sandwiching of SMA in between 2 CD34+ layers was still visible. Similarly, pericyte marker CD140b was also sandwiched between 2 CD34+ layers in capillaries. Thus, CD34 expression and SMA or pericyte marker expression were mutually exclusive, and these observations prompted the authors to conclude that ADSCs are likely CD34+CD31−SMA−CD140b− cells in the capillaries and in the adventitia of larger vessels. In addition, the authors also for the first time proposed that ADSCs might be VSCs, and ADSCs could differentiate into not only vascular cells (endothelial cells, SMCs, and pericytes) but also mesenchymal cells (adipocytes).

The second study dedicated to the histological analysis of ADSCs in the adipose tissue was published in 2010 [56]. In this study, CD34 was again used as the principal marker for ADSCs, and CD146, instead of CD140b, was used as the pericyte marker. The results were similar to those by Lin et al. [55] in that 2 concentric CD34+ rings and no co-expression of CD34 and CD146 were observed in blood vessels. In a flow cytometric analysis of freshly isolated adipose stromal vascular cells, a small population of CD34+CD146+ cells was detectable, but they were interpreted by the authors as endothelial progenitors, not ADSCs. Regardless, in agreement with Lin et al. [55], the CD34+CD146− adventitial cells were interpreted as ADSCs.

Another study investigating ADSCs within human adipose tissue also used CD34 as a defining marker, and for pericytes, it used CD140b and NG2 as markers [60]. Again, similar to the studies mentioned earlier, histological analysis showed no co-expression of CD34 and pericyte markers. In a flow cytometric analysis of freshly isolated adipose stromal vascular cells, 1.8% and 12.7% were found to express NG2 and CD140b, respectively, and less than 2% of these putative pericytes expressed CD34. In addition, a flow cytometric analysis of CD34-immunoselected adipose stromal vascular cells revealed that the expression of NG2 and CD140b increased 3.5- and 5-fold, respectively, during cell culture. Thus, in the Discussion section, the authors questioned the accuracy of the pericyte-as-ADSC theory as put forth by Traktuev et al. [59].

The most recent study [57] on the histological analysis of ADSCs in adipose tissue was published from the same lab as Crisan et al. [53], who contended that MSCs in most tissues, including the adipose, were pericytes. However, blood vessels that stained for CD34 and CD146 were substantially different between these 2 studies (see discussion in the "Pericytes vascular stem cells" section). While reasons for the discrepancy remain unknown, the new study appears to be more convincing, because it is dedicated to the analysis of ADSCs within adipose tissue (as opposed to MSCs in several different tissues) and its data are mostly consistent with other dedicated ADSC studies. Specifically, while still contending that their previously identified CD34−CD146+ cells were pericytes, the new study demonstrated the localization of CD34+CD146− cells in the adventitia. Thus, all 3 dedicated ADSC histological studies reached the same conclusion that CD34+ ADSCs reside in the adventitia and do not express pericyte markers [55–57].

In a study that was only recently published online [71], 3 vascular cell subsets, namely, CD34+CD146− ADSCs, CD34−CD146+ pericytes, and CD34+CD146+ endothelial cells, were isolated from human adipose tissue. Under MSC culture conditions, ADSCs, but not pericytes, were found to differentiate into MSCs as determined by the expression of several MSC markers. In addition, ADSCs were more clonogenic than pericytes, and in agreement with previous studies [57,60,72], propagation of ADSCs in culture resulted in down-regulation of CD34 and up-regulation of pericyte markers.

Pericytes or Adventitial Progenitor Cells

In addition to having the tri-lineage differentiation potential as mentioned earlier, pericytes are also known to possibly possess neurogenic differentiation potentials. However, in a recent review article, Goldberg and Hirschi [73] questioned the validity of such a theory:"Serving a contractile role to regulate blood flow, physiologically pericytes are not stem cells in the adult brain, and should not be confused with other perivascular progenitor cell types. Of the markers used to identify pericytes, including smooth muscle α-actin, desmin, NG-2, PDGFRβ, aminopeptidase A and N, and RGS5, none are entirely specific, and none recognize all pericytes."

This comment not only raises concern about the reliance of the so-called pericyte-specific markers but also touches on an important and fundamental question: How can a functionally sophisticated (and thus highly differentiated) cell still possess a large capacity for differentiation? Indeed, pericytes' functions are more than blood flow regulation, but as diverse as performing vessel stabilization, vascular tone regulation, vessel permeability regulation, local tissue homeostasis maintenance, macrophage-like properties, immunologic defense, coagulation intervention, and control of the quiescent and angiogenic stages of blood vessels [31]. Thus, with these large degrees of functional diversity and sophistication, how can pericytes still maintain a large capacity for differentiation, including the ability to become many different cell types with each having its own sophisticated functions, for example, osteoblasts, Leydig cells, neurons, chondrocytes, and adipocytes?

On the other hand, APCs are not known to have any specific physiological function and are, thus, developmentally more akin to primitive cells whose primary role is serving as progenitors for various specialized cell types. In the capillary, which lacks a defined adventitia, the APC equivalent still exists, as evidenced by the presence of the same CD34 + CD31 − CD140b − SMA − cells in both the adventitia and the capillary [5,55]. Thus, it has been postulated that, within the vasculature, APCs could differentiate into pericytes (CD34 − CD31 − CD140b + SMA −), endothelial cells (CD34 + CD31 + CD140b − SMA −), and SMCs (CD34 − CD31 − CD140b − SMA +); and during tissue expansion or repair, APCs could also differentiate into tissue-specific cell types (eg, muscle and fat) [5,55].

While this review clearly favors APCs over pericytes as the likely VSC candidate, it should be pointed out that currently the opposite view (ie, pericytes as VSCs) is more prevalent, and many excellent reviews, including a recent one, have discussed this issue extensively [74].

Differentiation of Vascular Stem Cells into Local Tissue Cells

As stated earlier, the role of MSCs has been proposed as providing tissue-specific cells for the normal turnover and maintenance of mesenchymal tissues [4]. Thus, if VSCs are indeed MSCs, then they need to be able to differentiate into local mesenchymal tissue cells, in addition to their expected ability to become vascular cells (endothelial cells and SMCs). However, even in the MSC field—not to mention the more recent VSC field—the great majority of studies in this regard are in vitro; for example, adipogenic differentiation of cultured MSCs. Nevertheless, in the ADSC field, 2 studies have come close to being in vivo—both employing the same strategy of transplanting cultured human ADSCs subcutaneously into nude mice and visualizing the formation of adipocytes from the transplanted cells [75,76]. Although the use of cultured ADSCs is in vitro, their transplantation into the subcutaneous space does provide an environment that is natural to ADSCs. Thus, under this semi in vivo condition, VSCs were shown to differentiate into the mesenchymal cell type (adipocyte) of their tissue origin.

Paracrine Effects of Vascular Stem Cells on Local Tissues

The ability to differentiate into various cell types has been considered one of the essential characteristics of MSCs. However, it is increasingly becoming clear that this capacity does not always account for MSC's therapeutic effects [9]. Indeed, in the ADSC field, the great majority of preclinical studies showed that ADSCs exerted their therapeutic effects through paracrine actions [77]. Thus, in their native environment, VSCs could possibly participate in the maintenance and/or expansion of mesenchymal tissues by the secretion of growth factors and cytokines. An example can be found in one of the 2 studies mentioned earlier, in which the transplanted ADSCs not only differentiated into adipocytes but also induced de novo adipogenesis in the host tissue [75].

Concluding Remarks

As defined earlier, VSCs reside within the vessel wall and can differentiate into all cell types that constitute a functional blood vessel. They can probably also differentiate into specific mesenchymal cells of their native tissue, or they can induce the replication/differentiation of such cells via growth factor secretion. Consistent with these proposed VSC characteristics, APCs are the most likely candidates for being VSCs. In culture, they spontaneously differentiate into cells that express pericyte markers; they can also be induced to differentiate into endothelial cells and SMCs. In addition, they have also been induced to differentiate into bone, cartilage, and fat cells. Thus, in vitro, APCs fulfill all criteria for being VSCs. In vivo, the evidence is still limited and will require further investigations.

Importantly, it should be pointed out that the VSC field is only beginning to emerge, and, thus, many issues regarding VSC's identity and function remain poorly understood. In fact, even after decades of intensive research, MSC, which is suggested to be VSC in this review, is still having many outstanding issues of its own. And, on top of this, likewise decades-long intensive pericyte research has not been able resolve the identity issue. Thus, in this review, neither the suggestion that VSCs are MSCs nor the argument that pericytes cannot be VSCs is set in stone. Rather, they are intended to encourage further discussion and research on these important yet controversial subjects.

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References

1. Tang Z, A Wang, F Yuan, Z Yan, B Liu, JS Chu, JA Helms and S Li. (2012). Differentiation of multipotent vascular stem cells contributes to vascular diseases. Nat Commun 3:875.
2. Adams RH and K Alitalo. (2007). Molecular regulation of angiogenesis and lymphangiogenesis. Nat Rev Mol Cell Biol 8:464–478.
3. Bautch VL. (2011). Stem cells and the vasculature. Nat Med 17:1437–1443.
4. Caplan AI. (2005). Review: mesenchymal stem cells: cell-based reconstructive therapy in orthopedics. Tissue Eng 11:1198–1211.
5. Lin CS, ZC Xin, CH Deng, H Ning, G Lin and TF Lue. (2010). Defining adipose tissue-derived stem cells in tissue and in culture. Histol Histopathol 25:807–815.
6. Lin G, Z Xin, H Zhang, L Banie, G Wang, X Qiu, H Ning, TF Lue and CS Lin. (2012). Identification of active and quiescent adipose vascular stromal cells. Cytotherapy 14:240–246.
7. Caplan AI. (2008). All MSCs are pericytes? Cell Stem Cell 3:229–230.
8. da Silva Meirelles L, AI Caplan and NB Nardi. (2008). In search of the in vivo identity of mesenchymal stem cells. Stem Cells (Dayton, Ohio) 26:2287–2299.
9. Prockop DJ. (2009). Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. Mol Ther 17:939–946.
10. Nico B, G de Falco, A Vacca, L Roncali and D Ribatti. (2001). In vivo absence of synergism between fibroblast growth factor-2 and vascular endothelial growth factor. J Hematother Stem Cell Res 10:905–912.
11. English D. (2002). Vascular stem cell transplantation: the next phase of hematotherapy? J Hematother Stem Cell Res 11:1.
12. Asahara T and JM Isner. (2002). Endothelial progenitor cells for vascular regeneration. J Hematother Stem Cell Res 11:171–178.
13. Tepper OM, RD Galiano, C Kalka and GC Gurtner. (2003). Endothelial progenitor cells: the promise of vascular stem cells for plastic surgery. Plast Reconstr Surg 111:846–854.
14. Stitt AW, CL O'Neill, MT O'Doherty, DB Archer, TA Gardiner and RJ Medina. (2011). Vascular stem cells and ischaemic retinopathies. Prog Retin Eye Res 30:149–166.
15. Fadini GP, P Madeddu, J Waltenberger and P Fiorina. (2012). Vascular stem and progenitor cells in diabetic complications. Exp Diabetes Res 2012:580343.
16. Keats EC and ZA Khan. (2012). Vascular stem cells in diabetic complications: evidence for a role in the pathogenesis and the therapeutic promise. Cardiovasc Diabetol 11:37.
17. Prokopi M, G Pula, U Mayr, C Devue, J Gallagher, Q Xiao, CM Boulanger, N Westwood, C Urbich, et al. (2009). Proteomic analysis reveals presence of platelet microparticles in endothelial progenitor cell cultures. Blood 114:723–732.
18. Yoder MC. (2010). Is endothelium the origin of endothelial progenitor cells? Arterioscler Thromb Vasc Biol 30:1094–1103.
19. Richardson MR and MC Yoder. (2011). Endothelial progenitor cells: quo vadis? J Mol Cell Cardiol 50:266–272.
20. Havelka GE and MR Kibbe. (2011). The vascular adventitia: its role in the arterial injury response. Vasc Endovascr Surg 45:381–390.
21. Majesky MW, XR Dong, V Hoglund, WM Mahoney, Jr., and G Daum. (2011). The adventitia: a dynamic interface containing resident progenitor cells. Arterioscler Thromb Vasc Biol 31:1530–1539.
22. Kovacic JC and M Boehm. (2009). Resident vascular progenitor cells: an emerging role for non-terminally differentiated vessel-resident cells in vascular biology. Stem Cell Res 2:2–15.
23. Pacilli A and G Pasquinelli. (2009). Vascular wall resident progenitor cells: a review. Exp Cell Res 315:901–914.
24. Tilki D, HP Hohn, B Ergun, S Rafii and S Ergun. (2009). Emerging biology of vascular wall progenitor cells in health and disease. Trends Mol Med 15:501–509.
25. Psaltis PJ, A Harbuzariu, S Delacroix, EW Holroyd and RD Simari. (2011). Resident vascular progenitor cells—diverse origins, phenotype, and function. J Cardiovasc Transl Res 4:161–176.
26. Melero-Martin JM and AC Dudley. (2011). Concise review: vascular stem cells and tumor angiogenesis. Stem Cells (Dayton, Ohio) 29:163–168.
27. Ergun S, D Tilki and D Klein. (2011). Vascular wall as a reservoir for different types of stem and progenitor cells. Antioxid Redox Signal 15:981–995.
28. Torsney E and Q Xu. (2011). Resident vascular progenitor cells. J Mol Cell Cardiol 50:304–311.
29. Bostrom KI, A Garfinkel, Y Yao and M Jumabay. (2012). Concise review: applying stem cell biology to vascular structures. Stem Cells (Dayton, Ohio) 30:386–391.
30. Zimmermann KW. (1923). Der feinere bau der blutcapillares. Z Anat Entwicklungsgesch 68:3–109.
31. Diaz-Flores L, R Gutierrez, JF Madrid, H Varela, F Valladares, E Acosta, P Martin-Vasallo and L Diaz-Flores, Jr. (2009). Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. Histol Histopathol 24:909–969.
32. Dore-Duffy P and K Cleary. (2011). Morphology and properties of pericytes. Methods Mol Biol 686:49–68.
33. Armulik A, G Genove and C Betsholtz. (2011). Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Dev Cell 21:193–215.
34. Schor AM, TD Allen, AE Canfield, P Sloan and SL Schor. (1990). Pericytes derived from the retinal microvasculature undergo calcification in vitro. J Cell Sci 97 (Pt 3):449–461.
35. Doherty MJ, BA Ashton, S Walsh, JN Beresford, ME Grant and AE Canfield. (1998). Vascular pericytes express osteogenic potential in vitro and in vivo. J Bone Miner Res 13:828–838.
36. Farrington-Rock C, NJ Crofts, MJ Doherty, BA Ashton, C Griffin-Jones and AE Canfield. (2004). Chondrogenic and adipogenic potential of microvascular pericytes. Circulation 110:2226–2232.
37. Diaz-Flores L, R Gutierrez, A Lopez-Alonso, R Gonzalez and H Varela. (1992). Pericytes as a supplementary source of osteoblasts in periosteal osteogenesis. Clin Orthop Relat Res 280–286.
38. Brighton CT, DG Lorich, R Kupcha, TM Reilly, AR Jones and RA Woodbury, 2nd. (1992). The pericyte as a possible osteoblast progenitor cell. Clin Orthop Relat Res 287–299.
39. Tigges U, JV Welser-Alves, A Boroujerdi and R Milner. (2012). A novel and simple method for culturing pericytes from mouse brain. Microvasc Res 84:74–80.
40. Balabanov R and P Dore-Duffy. (1998). Role of the CNS microvascular pericyte in the blood-brain barrier. J Neurosci Res 53:637–644.

41. Bostrom K, KE Watson, S Horn, C Wortham, IM Herman and LL Demer. (1993). Bone morphogenetic protein expression in human atherosclerotic lesions. J Clin Invest 91:1800–1809.
42. Watson KE, K Bostrom, R Ravindranath, T Lam, B Norton and LL Demer. (1994). TGF-beta 1 and 25-hydroxycholesterol stimulate osteoblast-like vascular cells to calcify. J Clin Invest 93:2106–2113.
43. Tintut Y, Z Alfonso, T Saini, K Radcliff, K Watson, K Bostrom and LL Demer. (2003). Multilineage potential of cells from the artery wall. Circulation 108:2505–2510.
44. Abedin M, Y Tintut and LL Demer. (2004). Mesenchymal stem cells and the artery wall. Circ Res 95:671–676.
45. Demer LL and Y Tintut. (2005). Return to ectopia: stem cells in the artery wall. Arterioscler Thromb Vasc Biol 25:1307–1308.
46. Sage A, Y Tintut, A Garfinkel and L Demer. (2009). Systems biology of vascular calcification. Trends Cardiovasc Med 19:118–123.
47. Sage AP, Y Tintut and LL Demer. (2010). Regulatory mechanisms in vascular calcification. Nat Rev Cardiol 7:528–536.
48. Davidoff MS, R Middendorff, G Enikolopov, D Riethmacher, AF Holstein and D Muller. (2004). Progenitor cells of the testosterone-producing Leydig cells revealed. J Cell Biol 167:935–944.
49. Chen H, RS Ge and BR Zirkin. (2009). Leydig cells: From stem cells to aging. Mol Cell Endocrinol 306:9–16.
50. Chen H, E Stanley, S Jin and BR Zirkin. (2010). Stem Leydig cells: from fetal to aged animals. Birth Defects Res C Embryo Today 90:272–283.
51. Zirkin BR. (2010). Where do adult Leydig cells come from? Biol Reprod 82:1019–1020.
52. Covas DT, RA Panepucci, AM Fontes, WA Silva, Jr., MD Orellana, MC Freitas, L Neder, AR Santos, LC Peres, MC Jamur and MA Zago. (2008). Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts. Exp Hematol 36:642–654.
53. Crisan M, S Yap, L Casteilla, CW Chen, M Corselli, TS Park, G Andriolo, B Sun, B Zheng, et al. (2008). A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3:301–313.
54. Pusztaszeri MP, W Seelentag and FT Bosman. (2006). Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. J Histochem Cytochem 54:385–395.
55. Lin G, M Garcia, H Ning, L Banie, YL Guo, TF Lue and CS Lin. (2008). Defining stem and progenitor cells within adipose tissue. Stem Cells Dev 17:1053–1063.
56. Zimmerlin L, VS Donnenberg, ME Pfeifer, EM Meyer, B Peault, JP Rubin and AD Donnenberg. (2010). Stromal vascular progenitors in adult human adipose tissue. Cytometry A 77:22–30.
57. Corselli M, CW Chen, B Sun, S Yap, JP Rubin and B Peault. (2012). The tunica adventitia of human arteries and veins as a source of mesenchymal stem cells. Stem Cells Dev 21:1299–1308.
58. Amos PJ, H Shang, AM Bailey, A Taylor, AJ Katz and SM Peirce. (2008). IFATS collection: the role of human adipose-derived stromal cells in inflammatory microvascular remodeling and evidence of a perivascular phenotype. Stem Cells (Dayton, Ohio) 26:2682–2690.
59. Traktuev DO, S Merfeld-Clauss, J Li, M Kolonin, W Arap, R Pasqualini, BH Johnstone and KL March. (2008). A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. Circ Res 102:77–85.
60. Maumus M, JA Peyrafitte, R D'Angelo, C Fournier-Wirth, A Bouloumie, L Casteilla, C Sengenes and P Bourin. (2011). Native human adipose stromal cells: localization, morphology and phenotype. Int J Obes (Lond) 35:1141–1153.
61. Zengin E, F Chalajour, UM Gehling, WD Ito, H Treede, H Lauke, J Weil, H Reichenspurner, N Kilic and S Ergun. (2006). Vascular wall resident progenitor cells: a source for postnatal vasculogenesis. Development 133:1543–1551.
62. Hu Y, Z Zhang, E Torsney, AR Afzal, F Davison, B Metzler and Q Xu. (2004). Abundant progenitor cells in the adventitia contribute to atherosclerosis of vein grafts in ApoE-deficient mice. J Clin Invest 113:1258–1265.
63. Torsney E, K Mandal, A Halliday, M Jahangiri and Q Xu. (2007). Characterisation of progenitor cells in human atherosclerotic vessels. Atherosclerosis 191:259–264.
64. Passman JN, XR Dong, SP Wu, CT Maguire, KA Hogan, VL Bautch and MW Majesky. (2008). A sonic hedgehog signaling domain in the arterial adventitia supports resident Sca1+ smooth muscle progenitor cells. Proc Natl Acad Sci U S A 105:9349–9354.
65. Yamashima T, AB Tonchev, IH Vachkov, BK Popivanova, T Seki, K Sawamoto and H Okano. (2004). Vascular adventitia generates neuronal progenitors in the monkey hippocampus after ischemia. Hippocampus 14:861–875.
66. Klein D, P Weisshardt, V Kleff, H Jastrow, HG Jakob and S Ergun. (2011). Vascular wall-resident CD44+ multipotent stem cells give rise to pericytes and smooth muscle cells and contribute to new vessel maturation. PLoS One 6:e20540.
67. Qian H, K Le Blanc and M Sigvardsson. (2012). Primary mesenchymal stem and progenitor cells from bone marrow lack expression of CD44 protein. J Biol Chem 287:25795–25807.
68. Pasquinelli G, PL Tazzari, C Vaselli, L Foroni, M Buzzi, G Storci, F Alviano, F Ricci, M Bonafe, et al. (2007). Thoracic aortas from multiorgan donors are suitable for obtaining resident angiogenic mesenchymal stromal cells. Stem Cells (Dayton, Ohio) 25:1627–1634.
69. Campagnolo P, D Cesselli, A Al Haj Zen, AP Beltrami, N Krankel, R Katare, G Angelini, C Emanueli and P Madeddu. (2010). Human adult vena saphena contains perivascular progenitor cells endowed with clonogenic and proangiogenic potential. Circulation 121:1735–1745.
70. Gimble JM, AJ Katz and BA Bunnell. (2007). Adipose-derived stem cells for regenerative medicine. Circ Res 100:1249–1260.
71. Braun J, A Kurtz, N Barutcu, J Bodo, A Thiel and J Dong. (2012). Concerted regulation of CD34 and CD105 accompanies MSC derivation from human adventitial stromal cells. Stem Cells Dev [Epub ahead of print]; DOI: 10.1089/scd.2012.0263.
72. Suga H, D Matsumoto, H Eto, K Inoue, N Aoi, H Kato, J Araki and K Yoshimura. (2009). Functional implications of CD34 expression in human adipose-derived stem/progenitor cells. Stem Cells Dev 18:1201–1210.
73. Goldberg JS and KK Hirschi. (2009). Diverse roles of the vasculature within the neural stem cell niche. Regen Med 4:879–897.
74. Peault B. (2012). Are mural cells guardians of stemness?: From pluri- to multipotency via vascular pericytes. Circulation 125:12–13.

75. Tsuji W, T Inamoto, H Yamashiro, T Ueno, H Kato, Y Kimura, Y Tabata and M Toi. (2009). Adipogenesis induced by human adipose tissue-derived stem cells. Tissue Eng Part A 15:83–93.
76. Zhang X, M Wu, W Zhang, J Shen and H Liu. (2010). Differentiation of human adipose-derived stem cells induced by recombinantly expressed fibroblast growth factor 10 in vitro and in vivo. In Vitro Cell Dev Biol Anim 46:60–71.
77. Lin CS and TF Lue. (2012). Adipose-derived stem cells: therapy through paracrine actions. In: *Stem Cells and Cancer Stem Cells*. Hayat MA, ed. Springer, New York, NY, Vol. 4, pp 203–216.

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