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REVIEW

Functional states of resident vascular stem cells and vascular remodeling

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Abstract **Recent evidence indicates that different types of vascular stem cells (VSCs) reside within the mural layers of arteries and veins. The precise identities of these resident VSCs are still unclear; generally, postnatal vasculature contains multilineage stem cells and vascular cell lineage-specific progenitor/stem cells which may participate in both vascular repair and lesion formation. However, the underlying mechanism remains poorly understood. In this review, we summarize the potential molecular mechanisms, which may control the quiescence and activation of resident VSCs and highlight a notion that the differential states of resident VSCs are directly linked to vascular repair or lesion formation.**

Keywords vascular stem cell, quiescence, activation, remodeling

Introduction

A rapidly growing body of evidence has indicated that different types of vascular stem cells (VSCs) reside within the mural layers of arteries and veins (Orlandi and Bennett, 2010; Bautch, 2011; Torsney and Xu, 2011; Tang et al., 2012). Generally speaking, postnatal vasculature contains multilineage stem cells and vascular cell lineage-specific progenitor/stem cells (Psaltis and Simari, 2015). These consist of multipotent mesenchymogenic populations; i.e., microvascular pericytes, multipotent vascular stem cells (MVSCs), and vascular wall mesenchymal stem cells (MSCs), and lineage-committed vascular progenitor/stem cells including endothelial progenitor cells (EPCs), smooth muscle progenitor cells (SPCs), adventitial macrophage progenitor cells (AMPCs), and hematopoietic progenitor cells (HSCs). However, there is no single marker for identifying these VSCs. While pericytes are defined by the presence of more than 2 markers including CD146, platelet derived growth factor receptor (PDGFR)-β, neuron-glia antigen 2 (NG2), CD13, SMα-actin (SMA), and desmin, MVSCs are identified by the expression of Sry-box (Sox)10, Sox17, neural filament-medium polypeptide (NFM) and S100β, and MSCs by the expression of CD44, CD90, CD105, CD45, and CD34. Several vascular cell populations expressing differential sets of unique protein markers are capable of differentiating to vascular cells such as smooth muscle cells (SMCs), endothelial cells (ECs), pericytes and macrophages. Since many of these VSCs are not strictly geared to differentiate into single type of vascular cells, they are broadly referred as to lineage-specific vascular progenitor cells/stem cells (VSCs). Of note, the lineage-specific VSCs express stem cell antigen (Sca)-1. However, the bona fide resident vascular progenitor cells have not been characterized. The biomarkers of resident VSCs that have been identified are summarized in Table 1. It is likely that resident VSCs participate in both vascular repair and lesion formation; however, the underlying mechanisms remain poorly understood.

Other studies of non-vascular adult SCs have raised a novel concept that adult SCs meet the requirement of adult organisms for survival by switching between functional states tuned to homeostasis, repair, and regeneration (Florian and Geiger, 2010; Tom and Cheung, 2012; Wabik and Jones, 2015). Adult SCs in a quiescent state are crucial for proper homeostasis and repair or regeneration; whereas the loss of quiescence may lead to spontaneous activation and premature differentiation of adult SCs, ultimately resulting in disease

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Table 1 Historical findings of adult resident VSCs found in different compartments of the vessel wall

Year	Authors	Source/species	Location	Population described	Isolation method	Progenitor cell differentiation potential	Cell marker expression summary	Summary
2001	Alessandri et al.	Human embryonic aorta rings	Adventitia	EPCs	Immunoselection of fresh digests	ECs	$CD31^+$ /$CD31^-$	$CD31^+$/$CD31^-$ cells were capable of differentiating into ECs and forming capillary-like structures.
2004	Hu et al.	Mouse thoracic aorta of $ApoE^{-/-}$ mice	Adventitia	Adventitial Sca-1^+ progenitors	Immunoselection of cultered tisue explant	SMCs	Sca-1^+, c-kit^+ /Lin^-	Sca-1^+ cells added to adventitial side of vein grafts in $ApoE^{-/-}$ mice, migration to intima observed.
2005	Covas et al.	Human saphenous vein-internal surface	Intima	MSCs	Culture of inner surface of veins	Osteogenic, chondrogenic, adipogenic	$CD13^+$, $CD29^+$, $CD44^+$, $CD54^+$, $CD90^+$, HLA $class^+$, HLA-DR	Human vein wall contains mesenchymal cells with marker profile and differentiation potential similar to other MSC sources such as bone marrow and umbilical vein.
2005	Ingram et al.	Human HUVEC/ HAEC	Intima	EPCs	Culture of HUVEC/HAEC	ECs	$CD31^+$, $CD141^+$, $CD105^+$, $CD145^+$, $DCD144^+$, vWF^+, Flk-1^+	EPCs isolated from HUVEC/HAEC had proliferative and clonogenic potential similar to blood derived EPC.
2005	Howson et al.	Rat Aorta	Mixed tissue source	PPCs	Immunoselection of fresh digests	Pericyte	CD34/Tie-2, NG2, nestin, PDGFR	Non-EC mesenchymal are capable of pericyte differentiation.
2006	Sainz et al.	Mouse aorta	Media	SP cells	Immunoselection of fresh digests	ECs, SMCs	Sca-1^+, c-kit (−low/) Lin-CD34 (−/low)	Media-derived SP cells were capable of differentiating into SMCs and ECs in response to PDGF/TGFB and VEGF, respectively.
2006	Zengin et al.	Human arteries and veins	Media-adventitia	MPCs	Arterial ring assays	ECs, hematopoietic and immune cells	$CD34^+$, $CD31^-$, $VEGFR2^+$, Tie-2	Vasculogenic zone between the media adventitia contain vascular wall progenitor cells. $CD34^+$/$CD31^-$ were capable of forming capillary like structures.
2007	Pasquinelli et al.	Human thoracic aorta	Media-adventitia	MSCs	Culture of whole arterial wall digests	ECs	$CD34^+$ or c-kit^+	Isolated cells from total vessel wall expressed mesenchymal markers ($CD44^+$, $CD90^+$, $CD105^+$) and stem cell markers (Oct4, c-kit, BCRP-1, interleukin-6) upon culture. MSCs displayed chondrogenic, adipogenic and leiomyogenic but less osteogenic potential, and formed capillary-like tubes, *in vitro*.
2007	Torsney et al.	Human aorta and mammary arteries	Atherosclerotic lesion/adventitia	VPCs	N/A. Immunostaining of neointimal lesion and adjacent aorta were conducted	N/A	CD34, c-kit, Sca-1	Progenitors identified within neointimal lesions and adventitia of human atherosclerotic vessels contained variable expression of CD34, Sca-1, c-kit and VEGFR2 markers, but no CD133 expression.
2007	Invernici et al.	Human fetal aorta	Adventitia	VPCs	Immunoselection of fresh digests	ECs, mural cells, and myocytes	$CD34^+$, $CD133^+$, $VEGFR2^+$, and desmin	VPCs formed by undifferentiated mesenchymal cells express endothelial and myogenic markers. VPCs can differentiate into ECs, mural cells or myocytes. VPCs can form 3D-cord-like vascular structures, *in vivo*. VPCs limproved neovascularization and muscular regeneration in a limb ischemic mouse model

(*Continued*)

Year	Authors	Source/species	Location	Population described	Isolation method	Progenitor cell differentiation potential	Cell marker expression summary	Summary
2008	Passman et al.	Mouse embryonic and adult arteries	Adventitia	Adventitial Sca-1$^+$ progenitors	Immunoselection of fresh digests	SMCs	Sca-1$^+$	Cells at media-adventitia interface have an Shh signaling domain. In Shh$^{-/-}$ mice adventitial Sca-1 cells were reduced. Sca-1$^+$ cells differentiated into SMCs.
2008	Hoshino et al.	Human pulmonary artery	Adventitia	MSCs	Culture of adventitial fibroblasts	Osteogenic, adipogenic, and leiomyogenic	Vimentin, collagen I, CD29, CD44, and CD105	Cultured vascular adventitial fibroblasts contain MSCs wich have adiopgenic and osteogenic potential.
2009	Liu et al.	Human blood and transplant atherosclerotic vessels	Intima	EOC	Culture of human mononuclear cells	ECs	ECs: eNOS, Tie-2, CD31, VECAD; Myeloid: CD14, CD68	Blood EOC outgrowths and ECs in neovessels of chimeric sex mismatched cardiac transplant atherosclerotic vessels express myeloid markers.
2009	Bearzi et al.	Human coronary arteries and capillaries	Intima, media, and adventitia	VPCs	Immunoselection of fresh digests	ECs, SMCs and angiogenic	VEGFR2$^+$, c-kit$^+$	VPCs, that were VEGFR2$^+$ and c-kit$^+$, had clonal and self-renewal capacity, and could differentiate toward EC and SMCs. VPCs also improved perfusion and generated new vessels in canine model of coronary stenosis.
2010	Pasquinelli et al.	Human arteries	Media-adventitia	MSCs	Culture of whole arterial wall digests	Adipogenic, chondrogenic, leiomyogenic	Oct-4, Stro-1, Sca-1, Notch-1, Mesenchymal markers (CD44, CD90, CD105, CD73, CD29, and CD166)	Oct-4, Stro-1, Sca-1, Notch-1 found in vasculogenic niche. Total vessel wall isolated showed expression of stem (Stro-1, otch-1, Oct-4) and MSC lineages (CD44, CD90, CD105, CD73, CD29 and CD166).
2010	Campagnolo et al.	Human saphenous vein	Mixed tissue source	SVPs	Immunoselection of fresh digests of total vessel wall	Pericyte	CD34, vimentin, desmin, NG2, PDGFRb, CD44, CD90, CD105, CD29, CD13, CD59, and CD73, Sox2	Cell isolates from total vessel wall contain CD34$^+$ /CD31$^-$ cells, which upon culture, express pericyte/mesenchymal markers. CD34$^+$/CD31$^-$ cells could integrate into vascular networks *in vitro* and *in vivo*.
2011	Klein et al.	Adult human arterial	Adventitia	MPSCs (i.e. MVSCs)	Immunoselection of fresh digests	SMCs	CD44$^+$, CD73$^+$, CD90, CD45$^-$, CD34$^-$	Mesenchymal stem cell can function as vasculogenic cells.
2012	Tsai et al.	Mouse thoracic aorta	Adventitia	Adventitial Sca-1$^+$ progenitors	Immunoselection of cultered tisue explant	ECs, SMCs	Sca-1$^+$	Sca-1$^+$ were able to differentiate into ECs and SMCs in response to VEGF or PDGF-BB stimulation, *in vitro*. *In vivo*, local application of VEGF to the adventitial side of the decellularized vessel increased re-endothelialization and reduced neointimal formation.
2012	Tang et al.	Mouse, rat carotid arteries, and human vessels	Media	MVSCs	Immunoselection of fresh digests and tissue explant method	SMCs, adipogenic, osteogenic, and chondrogenic, and neurogenic	SM-MHC-, Sox17, Sox10 S100β, NFM	MVSCs were small, migratory and proliferative SM-MHC cells, that had clonal and self-renewal capacity and differentiated into mesodermal and etodermal lineages, including SMCs. MVSCs were responsible for neointimal formation in endothelial denudation model.

(*Continued*)

Year	Authors	Source/species	Location	Population described	Isolation method	Progenitor cell differentiation potential	Cell marker expression summary	Summary
2012	Fang et al.	Human fetal aorta	Mixed tissue source	VESCs	Immunoselection of fresh digests	ECs, SMCs, osteogenic and adipogenic	Lin^-, $CD31^+$, $CD105^+$, $Sca\text{-}1^+$, $c\text{-}kit^+$	VESCs are clonal and have long-term self-renewal capacity. A single VESC can generate functional blood vessels, *in vivo.*
2012	Naito et al.	Mouse hindlimb vasculature and other tissues	Intima	SP ECs	Immunoselection of fresh digests	Angiogenic	Hoechst 33342/ $CD31^+$ /$CD45^-$	SP $CD31^-CD45^-$ Ecs were $Sca\text{-}1^+$, $VE\text{-}Cadh^+$, $Flk\text{-}1^+$, $CD133^+$, and CD34lo. They had greater clonogenic and angiogenic capacity than main population ECs and formed functional vessels *in vivo.*
2013	Chen et al.	Mouse thoracic aorta	Adventitia	Adventitial $Sca\text{-}1^+$ progenitors	Immunoselection of vein graft explant	SMCs, adipogenic, osteogenic, and chondrogenic	$Sca\text{-}1^+$	$Sca\text{-}1^+$ cells reside in close proximity to the vasa vasorum during pathological conditions of vein grafts. Adventitial $Sca\text{-}1^+$ progenitor cells can migrate across the vessel wall in response to SDF-1 for subsequent SMC differentiation, a process mediated by matrix protein/integrin interactions.
2013	Wong et al.	Mouse thoracic aorta	Adventitia	Adventitial $Sca\text{-}1^+$ progenitors	Immunoselection of cultered tisue explant	SMCs	$Sca\text{-}1^+$, Lin^-	Sirolimus-induced progenitor cell migration and differentiation into SMC via CXCR4 and epidermal growth factor receptor/extracellular signal–regulated kinase/β-catenin signal pathways, thus implicating a novel mechanism of restenosis formation after sirolimus-eluting stent treatment.
2015	Song et al.	Rat thoracic aorta	Media	RASMCs (i.e. MVSCs)	Immunoselection of fresh digests	SMCs, adipogenic, chondrogenic, and osteogenic	NFM, Sox10 and S100β	Traditionally cultured RASMCs probably result from the SMC differentiation of MVSCs. ROS is a negative regulator of MVSC differentiation into SMCs. Pla2g7 is a critical suppressor of MVSC differentiation into synthetic SMCs *in vitro*.

SMC indicates smooth muscle cell; EC, endothelial cell; EPC, endothelial progenitor cell; HAEC, human aorta endothelial cell; HUVEC, human umbilical vein endothelial cell; EOC, blood endothelial outgrowth cell; MPSC, multipotent stem cells; MVSC, multipotent vascular stem cell; MSC, mesenchymal stromal/ stem cell; MPC, macrophage precursor cell; SVF, stromal vascular fraction; SVP, saphenous vein progenitor cell; and VPCs, vascular progenitor cells; VESC, vascular endothelial stem cell; RASMC, rat aortic smooth muscle ell; ROS, reactive oxygen species.

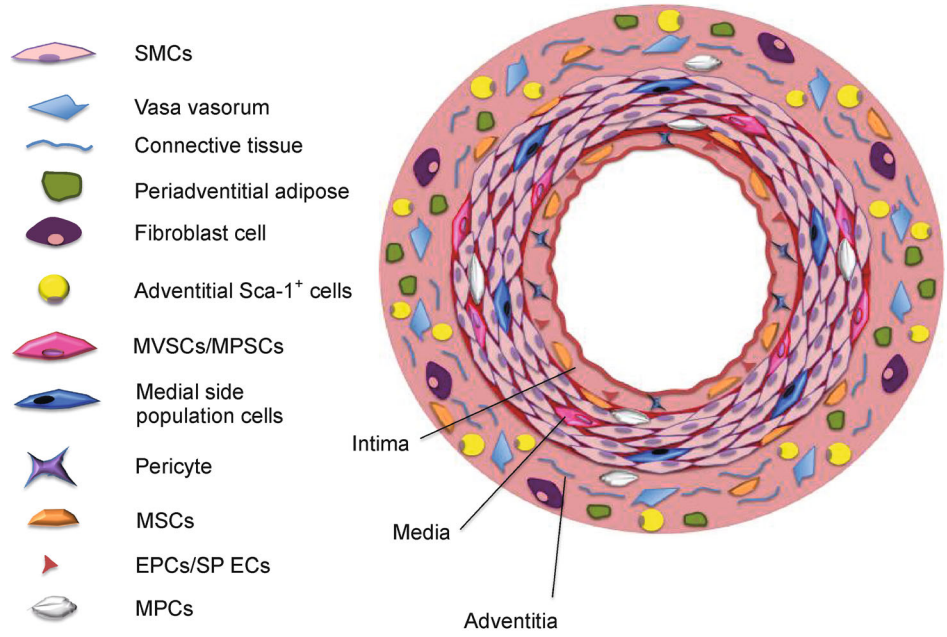

Figure 1 Diverse origin of adult resident VSCs in different compartments of the blood vessel. Distinct populations of vascular progenitor cells have been identified in the layers of the blood vessel. SMC indicates smooth muscle cell; MVSC, multipotent vascular stem cell; MPSC, multipotent stem cells; MSC, mesenchymal stromal/ stem cell; EPC, endothelial progenitor cell; SP EC, side population endothelial cell; MPC, macrophage precursor cell.

(Florian and Geiger, 2010; Tom and Cheung, 2012; Wabik and Jones, 2015). However, this theory has not been tested in resident VSCs. In this review, we summarized current literatures regarding the functional plasticity of adult SCs in tissue homeostasis, repair and regeneration and discuss the potential molecular mechanisms for controlling the functional states of resident VSCs, which are linked to vascular repair and lesion formation.

The functional plasticity of adult stem cells in non-vascular tissues or organs

Adult SC turnover has been established in a variety of tissues or organs. Under a normal and homeostatic state, adult SCs are maintained at a quiescent state characterized by their low RNA content, low proliferation markers, and low cellular turnover rate (Hüttmann et al., 2001; Fukada et al., 2007). In addition, one population of SCs behave so that one average, 50% of their progeny are themselves SCs (self-renewal) and 50% are differentiating cells; and the proliferating rate of SC "population asymmetry" is matched with the rate of loss of cells from differentiated lineage(s) it support (Wabik and Jones, 2015). However, following injuries or damage, SCs are activated and rapidly and reversibly switch to produce an excess of proliferating cells to repair or regenerate the loss tissue. Importantly, once activated, some adult SCs re-enter the quiescent state to maintain the SC population. Such switches of adult SCs between functional states, also known as functional plasticity, are critical for tissue homeostasis, repair, and regeneration. The functional plasticity of adult stem cells in non-vascular organs such as the squamous epithelia, the hair follicle, intestinal epithelium, bone marrow, male gonad, and neural tissues has been recently reviewed (Wabik and Jones, 2015).

These non-vascular adult SCs utilize several different strategies to maintain a homeostatic balance between self-renewal and differentiation. One strategy to control adult SC quiescence is by regulating cell-intrinsic signaling cascades, such as p53, FoxOs, hypoxia inducible factor (HIF)-1α, and CYLD-TRAF2-p38MAPK signaling pathways, or nuclear factor of activated T cells (NFAT) c1 signaling through ATM and mTOR (Li and Bhatia, 2011; Tesio et al., 2015). In addition to the cell-intrinsic mechanisms, the stem cell niche is also essential. A stem cell niche is a specific microenvironment that sustains and regulates stem cells. Stem cell niches have been identified for all types of adult stem cells in mammalian tissues, and contain other cell types for function and support. Quiescent adult SCs can respond to stimuli released by their niche environment allowing thereby causing their activation and cell cycle progression. Such extrinsic mechanisms involve angiopoientin-1, transforming growth factor (TGF)-β1, bone morphogenic protein (BMP), thrombopoietin (TPO), osteopontin, N-cadherin and integrin adhesion receptors, and Wnt/β-catenin signaling (Li and Bhatia, 2011; Tom and Cheung, 2012). Lastly, homeostasis of adult SCs can be maintained by asymmetric cell division which is a process whereby stem cells can give rise to two distinct daughter cells— one copy of the stem cell, the other of a differentiating cell. In the tissues with high cellular

turnover rate such as squamous SCs, SC asymmetrical division requires balancing the probabilities of three potential division outcomes: generating two SC, two differentiating cells, or one of each cell type. Lineage-tracing experiments conducted on squamous SCs indicate that their balanced stochastic fate is cell-intrinsic property rather than governed by external regulation; however, the mechanisms governing this population asymmetry have not been fully explored (Wabik and Jones, 2015). It has been speculated that cell polarity, is an important factor in asymmetric and symmetric cell division. A polarized is a cell in which its organelles, proteins, mRNAs, and/or miRNAs are distributed and maintained in a nonsymmetrical organization. This cell polarization can result from extracellular stimuli, which can induce the redistribution of cellular components to fulfill functional needs during adhesion, migration, or cell proliferation. Small RhoGTPase CDC42 has been shown to be an important factor in establishing polarity through interaction with the PAR polarity complex in a number of cell types ranging from the yeast to mammals (Florian and Geiger, 2010). Regardless of the approach for maintaining homeostasis, adult SC division is limited by a requirement to maintain a constant cell density within the niche.

Repair is also achieved by many different mechanisms such as mobilization of reserve adult SCs, SC plasticity and de-differentiation. These mechanisms are regulated by specific growth factors, which can drive their differentiation into various lineages. Following tissue damage, stem cells, such as HSCs, will exit quiescence thereby limiting their self-renewal capacity (Li and Bhatia, 2011; Tom and Cheung, 2012). An imbalance of stem cell self-renewal and differentiation will result in stem cell depletion, eventually leading to the premature activation and differentiation of HSCs, thereby causing hematological failure (Li and Bhatia, 2011). Given the role of HSC quiescence in homeostasis and repair, it is possible adult resident VSCs act in the same fashion. It is therefore proposed that the dysfunction of VSCs is essential in the pathogenesis of vascular disease. An active state of adult VSCs may be an indication of maladaptive vascular remodeling caused by the loss of quiescence and consequent activation of VSCs. Therefore, a quiescent status of adult resident VSCs may be critical for the repair of a damaged vasculature.

Diverse origins, phenotype, and function of resident VSCs

The current understanding of adult resident VSCs is somewhat rudimentary. Adult resident VSCs are believed to originate from embryogenesis. These cells are necessary for vascular development and remain within the vasculature after birth for post-natal growth, aging and disease (Psaltis and Simari, 2015). These cells remain quiescent until activated by vascular injury or disease. Given their close proximity, they are quickly recruited to the site of injury for self-renewal and differentiation (Psaltis et al., 2011). These stem/progenitor cells have very distinct phenotypes and appear in different compartments in the vessel wall – the intima, media and adventitia (Table 1). The characterization of these adult resident VSCs remains unclear, due to the discrepancies in multiple reports. However, what is clear is that these adult resident VSCs have the potential to differentiate to SMCs, ECs, and pericytes (Psaltis and Simari, 2015). This is evident by their upregulation lineage specific cell markers after stimulation with various growth factors and chemokines. In addition, these VSCs have adipogenic, chondrogenic, osteogenic, leiomyogenic and angiogenic potential (Psaltis and Simari, 2015). Although there are currently no established markers, Sca-1 is a primary marker used to identify adult resident VSCs in the murine vasculature. Multiple reports also indicate that these resident VSCs can contribute to vascular lesion formation rather than repair.

Adult resident VSCs have been found in multiple locations in the murine vasculature, including the tunica adventitia, tunica media, and tunica intima (Fig. 1). The tunica intima is the innermost layer of the blood vessel and is comprised one layer of ECs and a subendothelial layer consisting of delicate connective tissue. These structures are supported by internal elastic lamina, which separates the intimal layer from the medial layer. Recent evidence indicates the presence of VSCs in the intimal layer where they have the potential to repair damaged endothelium. These VSCs have been identified as SP ECs, EPCs, and MSCs (Table 1). The tunica media is the middle layer of the blood vessel and is comprised mainly of smooth muscle cells as well as elastic tissue. VSCs that have been identified in the medial layer include MVSCs, MPSCs, SP cells, and MSCs (Table 1). Tunica adventitia is the outermost layer of the blood vessel and is comprised of connective tissue, periadventitial adipose, fibroblasts, macrophages, vasa vasorum, and other cell types (Majesky et al., 2011). Several studies of animal models reveal VSCs in the adventitia, which can contribute to vascular pathologies such as atherosclerosis and restenosis. These VSCs have been identified as adventitial Sca-1$^+$ progenitors, MSCs, and MPCs (Table 1). Studies have also been conducted on whole tissue digests, which include the intima, media, and adventitial layer of the blood vessel. Consequently, the location of VSCs cannot be identified. VSCs that have been identified in whole tissue digests include PPCs, SVPs, and VESCs.

Current studies have focused on identifying the precise location where adult VSCs reside within the compartments of the vessel wall. Hu and his colleagues were the first to report of abundant progenitor cells expressing Sca-1$^+$, c-kit$^+$, Flk-1, and CD34$^+$ but not embryonic stem cell marker SSEA-1$^+$ in the aortic root adventitia in apolipoprotein E (ApoE)-deficient (*ApoE*$^{-/-}$) mice (Hu et al., 2004). Since the discovery of adventitial Sca-1$^+$ progenitors residing in the aortic root, many studies have focused on determining the origin, precise

locations, phenotype, and function of these VSCs. Interestingly, a significant population of Sca-1$^+$ cells was found close proximity to the vasa vasorum, within the remodeled adventitia (Chen et al., 2013). Campagnolo et al. (2010) also identified a new population of pericyte progenitor cells, which resides around adventitial vasa vasorum. These results suggest that the vasa vasorum may play a role in sustaining the survival of VSCs. The vasa vasorum is a network of small blood vessels that supply oxygen and nutrients the walls of arteries and blood vessels. It is possible that these stem/progenitor cells exist within these specialized compartments for survival via the uptake of growth factors and nutrients supplied by vasa vasorum (Kawabe and Hasebe, 2014). Importantly, their results identify yet another location within the adventitial layer of the aorta that harbors stem/progenitor cell populations. Previously mentioned, the aortic root adventitia had been established as a stem cell niche harboring an abundant source of Sca-1$^+$ progenitor cells (Hu et al., 2004). Also noteworthy, only $\approx$ 5% to 10% of the adventitial Sca-1$^+$ cells could form primary colonies varied in size and morphology, suggesting different subpopulations of Sca-1$^+$ progenitor cells. More studies will need to be conducted to characterize these vascular resident stem/progenitor cells; however, these discoveries highlight the importance of various VSCs in the murine vasculature and their participation in vascular remodeling.

Various methods have been used to determine the profile of adult VSCs mentioned in Table 1, including qRT-PCR, Western blot, and immunostaining. In addition, isolation of adult VSCs was performed by either immunoselection of cultured tissue explant or immunoselection of fresh digests. Different methods were performed for different studies, which may account for the variability in VSC profiles reported. It is not clear which method is more valid for evaluating cell phenotypes. However, what is clear is that there needs to be more overlap in the use of cell markers and the tests performed to fully characterize these isolated progenitor cells prior to stimulation and ultimately determine their cell lineage fate.

The quiescence and activation of resident VSCs

While the functional states of adult SCs in non-vascular tissues and organs have been evaluated, there are no reports regarding the functional states of adult resident VSCs in the vasculature. Most of the current studies of resident VSCs have focused on their identities and potential to be differentiated into vascular cells. Few have investigated the molecular pathways governing the quiescence and activation of adult resident VSCs. Nevertheless, several studies characterizing resident VSCs at the basal condition and differentiating states may provide some clues for understanding the significance of functional states of resident VSCs in the vasculature.

Potential intrinsic mechanisms regulating quiescence of VSCs

It is possible that aforementioned mechanisms that control non-vascular adult stem cell quiescence and activation may also be applicable to adult resident VSC quiescence and activation. For example, transcription factor p53 is a critical for maintaining HSC and NSC quiescence (Liu et al., 2009; van Os et al., 2009; Wang et al., 2009). The absence of p53 was also found to accelerate atherosclerosis by increasing cell proliferation *in vivo* (Guevara et al., 1999). Given that resident VSCs potentially contribute to atherosclerosis (Psaltis and Simari, 2015), it is possible that p53 deficiency results in the loss of VSC quiescence thereby impairing their self-renewal capacity, ultimately leading to exhaustion of the stem cell pool. Moreover, the loss of quiescence may lead to spontaneous activation and premature differentiation of VSCs, which can contribute to neointimal formation. The role of p21 was also evaluated in stem cell compartments such as HSCs and NSCs. Inhibition of p21 resulted in an increase in stem cell proliferation and in a decrease in the quiescent stem cell population (Cheng et al., 2000; Kippin et al., 2005). Because both p53 and p21 have been shown to regulate quiescence in HSCs and NSCs, whether VSCs also utilize the same pathways for their quiescent state is worthy to be determined.

Reactive oxygen species (ROS) is important in the self-renewal of stem cells. ROS in stem cells regulate expression of the transcription factors FoxOs and ATM, which in turn act to regulate ROS levels in stem cells and maintain stem cell quiescence (Li and Bhatia, 2011; Tom and Cheung, 2012;). Recently, we have also found that phospholipase A2, group 7 (Pla2g7) is a critical regulator in the maintenance of MVSCs via facilitation of endogenous ROS formation (Song et al., 2015). Of interest, undifferentiated MVSCs generated more ROS. Knockdown of Pla2g7 suppressed ROS formation in the MVSCs while enhancing SMC differentiation of MVSCs, suggesting that cultured synthetic VSMCs may be derived from SMC differentiation of MVSCs with ROS as a negative regulator. These novel findings revealed that Pla2g7-governed ROS is critical for the maintenance, and therefore, quiescent state of MVSCs.

The current body of evidence for intrinsic mechanisms that regulate VSC quiescence is promising. By exploring the intrinsic mechanisms that are already known to regulate non-vascular stem cells could provide a lead for investigating stem cells of vascular origin. Nevertheless, further studies will need to be conducted to determine the potential link between adult VSC quiescence and activation and vascular remodeling and disease.

Potential extrinsic mechanisms regulating quiescence of VSCs

Interactions of stem cells with the microenvironment are critical for the maintenance of HSC quiescence. TGF-β and bone morphogenic protein (BMP) produced by microenvironmental-supporting cells are important regulators of stem cell quiescence (Li and Bhatia, 2011; Tom and Cheung, 2012). TGF-β is a key negative regulator in HSC quiescence in vitro, and is hypothesized to be an important regulator of stem cell quiescence *in vivo* (Blank et al., 2008). TGF-β was also reported to be an important regulator in VSC differentiation to SMCs (Sainz et al., 2006; Tang et al., 2012) and BMP was shown to promote VSC differentiation of Sca-1$^+$ progenitors to osteogenic cells (Passman et al., 2008). Collectively, these results suggest that TGF-β and BMP may be causing VSC loss of quiescence, resulting in their rapid activation and differentiation.

The adhesion molecules N-Cadherin and β1-integrin are necessary for HSC anchoring to the microenvironment; however, they also play a role in HSC cycling (Zhang et al., 2003). N-Cadherin is present at the interface between HSCs and osteoblastic cells (Zhang et al., 2003). Interactions of angiopoietin-1 (Ang-1) with its receptor Tie-2 and thrombopoietin (TPO) with its receptor MPL promote stem cell quiescence and enhance HSC adhesion through β1-integrin and N-Cadherin receptors (Arai et al., 2004; Yoshihara et al., 2007). Therefore, β1-integrin and N-Cadherin may be key downstream targets of Tie2/Ang-1 and MPL/TPO signaling in HSCs. However, in adult resident VSCs, it appears as if N-Cadherin and β1-integrins play an opposite role. During vascular development and remodeling, SMCs exhibit very high rates of synthesis of extracellular matrix (ECM) components, including cadherins, and integrins, that make up a major portion of the blood vessel wall (Owens et al., 2004). These ECM proteins are important in maintaining tissue structure and cell function. Cells bind to the ECM via specific integrin receptors, and this binding can direct cell function. Chen *et al* explored collagen/integrin interactions in the activation and differentiation of adult resident VSCs to SMCs (Chen et al., 2013). Isolated adventitial Sca-1$^+$ progenitor cells from the adult vasculature were cultured in the presence of collagen IV for six days, which drove the upregulation of SMC gene expression markers (SM22α, CNN1, αSMA, and SM-MHC). The induction of SMC markers (CNN1 and SM22α) was also confirmed by immunofluorescence staining and Western blot. SMC differentiation resulted in a marked increase in the expression of several integrins, including α4, α5, and β1. Concomitantly, FAK was also activated, thereby supporting the involvement of integrins in the differentiation process. These results confirm the interaction of integrins, notably α4, α5, and β1, with collagen matrix proteins, are also critical in regulating the activation and differentiation of adult resident VSCs. While β1-integrins are involved in HSC quiescence, it appears that β1-integrins are also involved in adventitial Sca-1 activation and differentiation. Similar pathways were used however the outcomes were vastly different.

There is evidence that Wnt/β-catenin signaling in the microenvironment also plays a crucial role for maintaining HSCs in a quiescent state. Recent evidence also demonstrates that Wnt/β-catenin pathways regulate endothelial dysfunction and vascular smooth muscle cell proliferation and migration and thereby intimal thickening (Tsaousi et al., 2011). While there is no direct evidence of Wnt/β-catenin signaling involvement in the regulation of adult VSCs, it is possible that Wnt/β-catenin signaling is impairing the self-renewal capacity of VSCs. The dysfucntion of adult VSC can cause spontaneous activation and premature differentiation of VSCs to ECs and SMCs, thereby contributing to vascular remodeling and disease. Further studies will need to be conducted to elucidate the involvement of Wnt/β-catenin signaling in adult resident VSCs.

Cyclin-dependent kinase 8 (CDK8) has a conserved function in transcriptional reprogramming as part of the Mediator complex, and appears to regulate several signaling pathways that are critical for the control of stem cell pluripotency and turmorgenesis (Adler et al., 2012; Porter et al., 2012). CDK8 has been implicated in many molecular pathways including the Wnt/β-catenin pathway, p53 pathway and TGF-β signaling pathway(Cheng et al., 2000), which have been linked to stem cell quiescence and activation aforementioned. These results suggest that CDK8 may play a role in adult resident VSC quiescence and activation in vascular remodeling. Indeed, we have identified CDK8 as a critical regulator of VSC quiescence and activation. It is most likely that CDK8 is required for activated VSC proliferation and reprogramming to premature VSMC progenitors, which differentiate into synthetic VSMCs contributing to lesion formation [unpublished data].

Summary and future direction

There exist diverse origins of adult resident VSCs in different layers in blood vessel, which may contribute to vascular homeostasis and remodeling in a disease state. The pathophysiological consequences of resident VSC-mediated vascular remodeling are most likely dependent on their functional states. A quiescent state of resident VSCs may be critical for their self-renewal and generation of mature vascular progenitor cells for vascular homeostasis (Fig. 2). An active state of resident VSCs may be needed for providing extra vascular progenitor cells for repair in response to vascular injury; however, abnormal activation of VSCs may lead to generation of premature vascular progenitor cells leading to maladaptive vascular remodeling which eventually causes vascular dysfunction and disease (Fig. 2). Many things regarding resident VSCs and progenitor cells remain to be addressed, such as their profile, location, method of isolation,

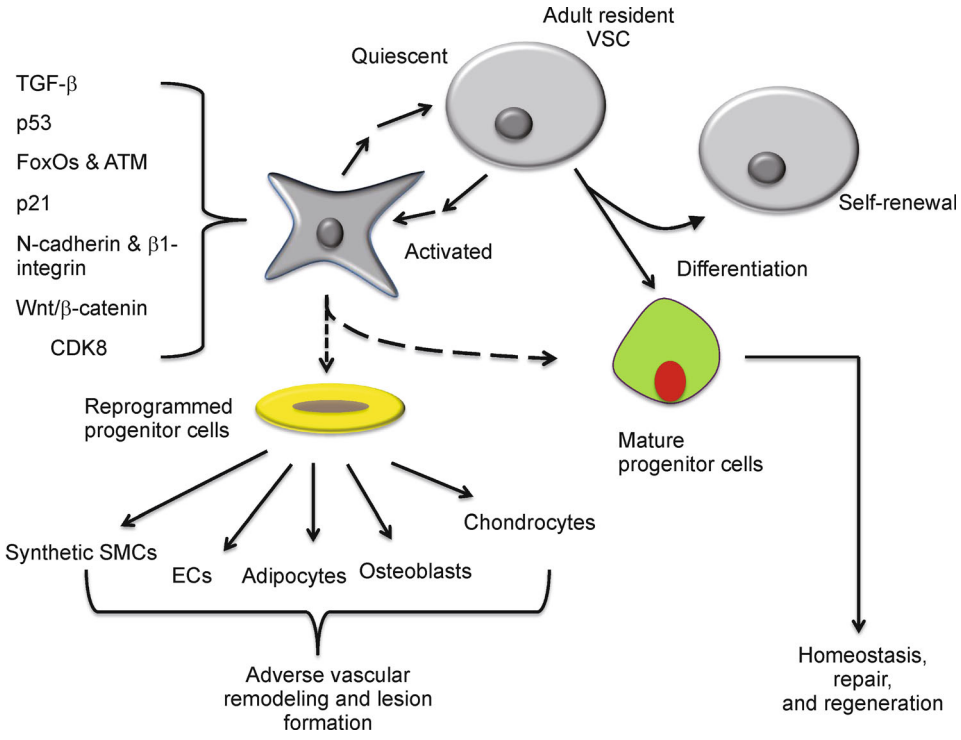

Figure 2 Adult VSC quiescence and activation. Adult VSCs at a quiescent state maintain their self-renewal and mature progenitor differentiation, which are required for vascular homeostasis, repair or regeneration. In response to injury, quiescent adult VSCs may be transformed into active VSCs, which are also precursors for mature progenitor cells for vascular repair. However, loss of the quiescence due to either dysregulated intrinsic or extrinsic signaling results in the abnormal activation and reprogramming of VSCs thereby leading to generation of premature progenitor cells and subsequent multiple lineage differentiation. This differentiation mostly likely produces various types of premature cells, such as synthetic SMCs, dysfunctional ECs, adipocytes, osteoblasts, and chondrocytes, contributing to maladaptive vascular remodeling and eventually causing vascular disease.

culture conditions, and animal injury models, as well as mechanisms which control their differentiation and function. A full evaluation of adult resident VSC contribution to vascular remodeling cannot be performed without considering all of these factors. Most importantly, addressing these aspects may connect with the discrepancies in the multiple reports. Further investigation of molecular mechanisms for controlling the functional states of resident VSCs may uncover the nature of resident VSCs per se as well as the precise contributions of VSC-mediated vascular lesion formation and repair.

Nonstandard abbreviations and acronyms

CYLD	Cylindromatosis
FoxO	Forkhead box O
ATM	Ataxia telangiectasia mutated
Wnt	Wingless-type MMTV
TRAF2	TNF receptor-associated factor 2
Rho	Rhodamine
PAR	Partition protein
SSEA-1	Stage-specific embryonic antigen
CNN1	Calponin 1
SM-MHC	Smooth muscle myosin heavy chain
αSMA	α smooth muscle actin
ApoE	Apolipoprotein E
SDF-1	Stromal cell derived factor-1
HUVEC	Human umbilical vein endothelial cells
HAEC	Human aortic endothelial cells
SP	Side population
BMP	Bone morphogenic protein
PDGF	Platelet-derived growth factor
PDGFRβ	Platelet derived growth factor receptor β
TGFB	Transforming growth factor beta
VEGF	Vascular endothelial growth factor
VEGFR2	Vascular endothelial growth factor receptor 2
Shh	Sonic hedgehog
CXCR4	C-X-C Chemokine receptor type 4
Pla2g7	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)
Oct-4	Octamer-binding transcription factor 4
BCRP-1	Breakpoint Cluster Region Pseudogene 1
Flk-1	Fetal liver kinase 1
ROS	Reactive oxygen species
CDK8	Cyclin-dependent kinase 8
ECM	Extracellular matrix
Sca-1	Stem cell antigen-1

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Compliance with ethics guidelines

This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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