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Commentary

Progenitor cell-derived smooth muscle cells in vascular disease

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ABSTRACT

Accumulation of vascular smooth muscle cells (VSMCs) in the tunica intima plays a major role in the pathogenesis of atherosclerosis and restenosis following endovascular procedures. Arterial VSMCs are heterogeneous even in the normal vessel wall and display different phenotypes in physiological and pathological conditions. In the classical paradigm, vascular wall injury induces VSMC de-differentiation, proliferation and migration from the media into the intima in response to growth factors and proteolytic agents. Accordingly, VSMCs in atherosclerotic plaques and in restenosis display a de-differentiated or 'synthetic' phenotype compared to a 'contractile' phenotype in the normal media. In contrast, recent studies have identified bone marrow and peripheral blood-derived endothelial and VSMC progenitors that may contribute to intimal formation in atherosclerosis, after arterial injury and in transplant atherosclerosis. The precise frequency of these bone marrow-derived vascular precursor cells is controversial and their role is unknown. In addition, additional data support the presence of a resident progenitor cell subpopulation and its involvement in the response of the adult arterial wall to damage or ischemia. This review will examine the evidence for and the putative role of progenitor cell-derived VSMCs in arterial disease, a necessary prerequisite before deciding whether progenitor cells are therapeutic targets in vascular disease.

1. Introduction

Atherosclerosis is a chronic inflammatory disease, in which risk factors result in dysfunction and damage to the arterial endothelium [1–3]. Endothelial damage permits migration of inflammatory cells and accumulation of lipid, followed by proliferation and migration of vascular smooth muscle cells (VSMCs) from the media into the intima. Following arterial injury to either normal or diseased vessels, proliferation and subsequent accumulation of VSMCs results in further intimal expansion, which can result in restenosis [4]. The classical paradigm of atherosclerosis suggests that accumulation of VSMCs in the tunica intima plays a crucial role in the pathogenesis of atherosclerosis and restenosis following angioplasty or stenting [1–3]. In the original 'response to injury' hypothesis of atherosclerosis, growth factors and proteolytic agents induce VSMC proliferation and migration from the tunica media into the intima, akin to the vessel wall response after mechanical injury [4]. During this process, VSMCs switch from a 'contractile' to a 'synthetic' phenotype, with reduced expression of typical VSMC contractile protein markers and an enhanced response to growth and chemotactic factors [5]. In atherosclerosis, VSMC accumulation in the fibrous cap is monoclonal or oligoclonal [6,7], implying that only a small number of medial VSMCs undergo proliferation. Subsequent studies examining telomere loss indicate that fibrous cap VSMCs have undergone 10–14 more population doublings than cells in the normal media [8], suggesting the existence of a resident arterial subpopulation that contributes to arterial healing in response to injury [9].

More recent studies have questioned the origin of VSMCs comprising atherosclerosis and neointima formation after injury or in transplant disease. In particular, studies in transplantation arteriopathy showed that recruitment of bone marrow or host-derived circulating precursors contributes to the intima after *in situ* SMC differentiation [10]. Similar studies in primary atherosclerosis and after arterial injury demonstrated bone marrow-derived VSMCs in both experimental animals and humans [11–13], although these findings have since been refuted [14,15]. Finally, the presence of cells expressing stem cell antigens in the normal arterial wall suggests the existence of resident progenitor cells capable of contributing to neointima formation [16,17]. Whilst most studies focused on uncovering their origin, the behavior of stem marker-expressing VSMCs and their contribution to vascular remodeling following acute and chronic damage remain largely unknown. In this review we will examine the evidence for arterial VSMC heterogeneity and

Abbreviations: VSMCs, vascular smooth muscle cells, stem cell antigen-1 (sca-1); α-SMA, alpha smooth muscle cell actin; SMMHC, smooth muscle cell myosin heavy chain.

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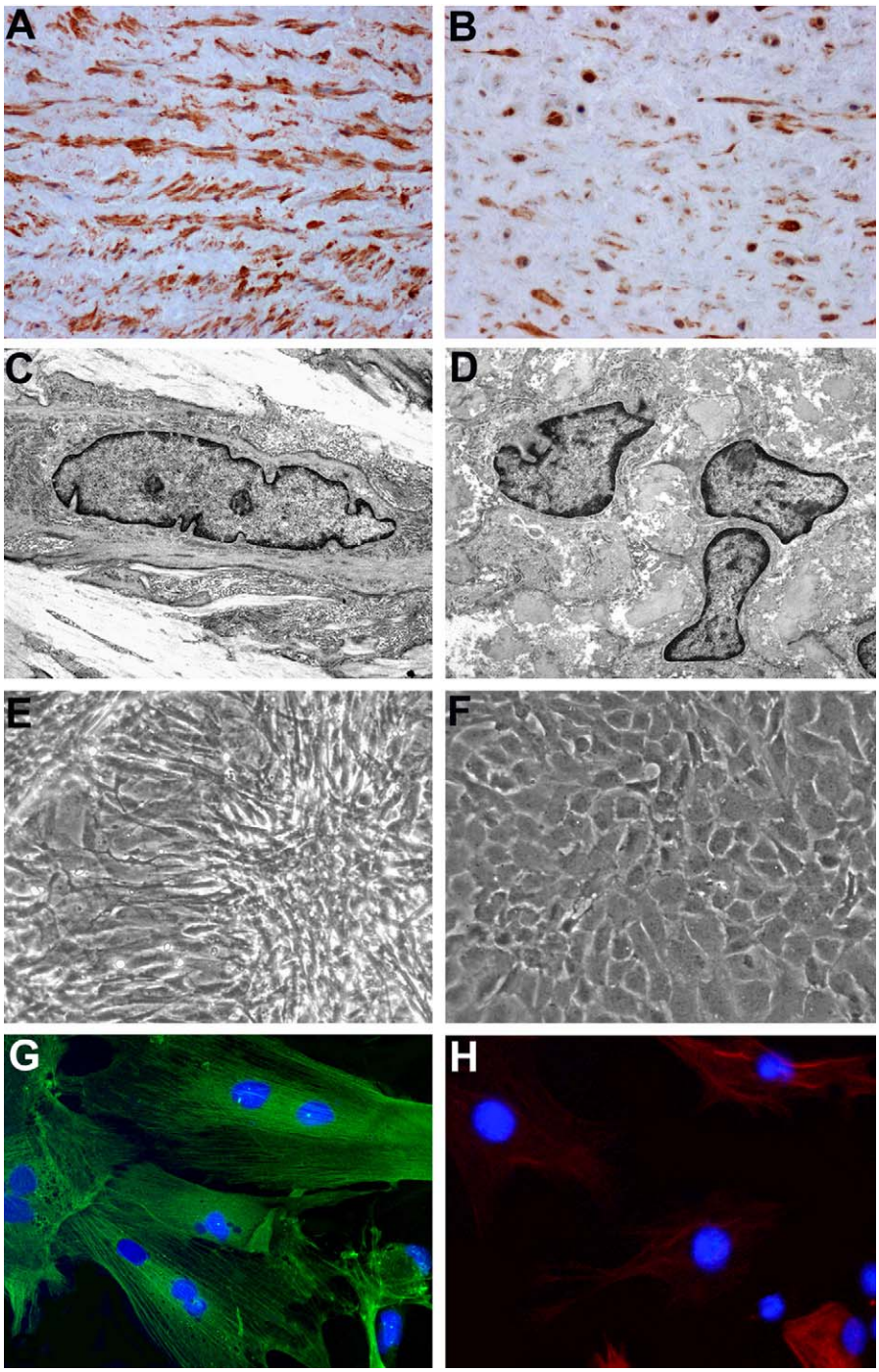

Fig. 1. Phenotypic heterogeneity of vascular smooth muscle cells. (A and B) Immunostaining of human aortic formalin-fixed sections stained with an antibody to anti-smooth muscle myosin heavy chain for VSMCs in (A) tunica media (B) diffuse intimal thickening in 54-year-old man. (C and D) Transmission electron photomicrographs showing (C) elongated rat aorta normal media VSMC SMC and (D) neointimal VSMCs SMCs fifteen days after ballooning. (E) Cultured rat aortic normal media VSMCs with the classical "hill-and-valley" confluent growth pattern, compared with (F) the monolayered and epithelioid appearance of neointimal VSMCs obtained fifteen days after ballooning. Immunofluorescence reveals that (G) rat aortic normal medial VSMCs display abundant typical α-smooth muscle actin cytoplasmic fibers, whereas they are reduced in (H) neointimal VSMCs. Original magnifications, A and B: 200×, C and D: 4000×, E and F: 100×, G and H: 400×.

the existence of VSMC progenitor cells and the contribution of stem cell-derived VSMCs to the development of vascular disease.

2. The heterogeneity of vascular smooth muscle cells

2.1. Vascular smooth muscle cells display different phenotypes

VSMCs within the normal media are heterogeneous. VSMC heterogeneity is manifest by 'contractile' and 'synthetic' phenotypes [18–20], which are typical of VSMCs of the normal and pathologic arteries, respectively (Fig. 1). These phenotypes are also seen *in vitro*; for example two populations are obtained from the post-injury rat aorta and carotid artery: a spindle-shaped phenotype, with the classic "hill-and-valley" growth pattern typical of cultured normal medial VSMCs and an epithelioid phenotype, with cells growing in a monolayer with a cobblestone morphology at confluence that is typically isolated from the neointimal tissue fifteen days after balloon injury [20,21]. Clones

with a spindle-shaped and epithelioid phenotype can also be obtained from the normal media of rat [22], pig [23] and human vessels [24] and phenotypic differences are also found in VSMCs cultured from aortas obtained from animals of different ages [22]. The contractile and synthetic phenotypes are not permanent and phenotypic modulation occurs in culture, particularly under the influence of growth factors such as PDGF-BB or FGF-2, including in human VSMCs [23,24], and *in vivo*. Modulation changes cell behavior, as phenotypically-regulated activation of proteins and receptors regulates VSMC proliferation, migration and survival. For example, rat aortic epithelioid neointimal VSMCs proliferate faster and are more sensitive to apoptosis induced by retinoic acid and *cis*-platinum [25] and a phenotype-regulated increase in expression of the EGF receptor results in cell cycle arrest through activation of a specific protein kinase pathway [26]. Similarly, phenotype-regulated expression of NF-κB is linked to the different apoptotic susceptibility of epithelioid VSMCs [27] and phenotypic heterogeneity is accompanied by a different capacity to accumulate cholesteryl esters [28]. Finally, differential expression of α-integrins in phenotypically distinct VSMC populations regulates their behavior in a collagen matrix [29] and differential expression of genes also influences vascular motility [30].

2.2. Heterogeneity and the origin of vascular smooth muscle cells

A possible explanation of the heterogeneity of VSMCs in adult vessels can be found in embryologic vascular development. During vasculogenesis, VSMCs originate from different sources depending on the vessel type, including mesoderm [31], neurectoderm [32], epicardium (for coronary arteries [33]) and, more rarely, endothelium [34]. It is thus possible that the various VSMC phenotypes can arise from distinct lineages. In the chick embryo aorta, spindle-shaped and epithelioid phenotypes that respond differently to TGF-β have been isolated from two distinct regions of the artery, which differ in their embryologic origin, namely, the mesoderm and neural crest [35,36]. If VSMCs from different embryological origins retain their different responses to microenviromental changes and to arterial damage, this may underlie different contributions to the development of vascular lesions [34].

3. Vascular smooth muscle progenitors

VSMC progenitor cells have been identified in the bone marrow (multipotent vascular stem cell progenitors and mesenchymal stem cells), in the circulation (circulating VSMC progenitor cells), in the vessel wall (resident VSMC progenitor cells and mesangioblasts) and various extravascular sites (extravascular, non-bone marrow progenitor cells; Fig. 2). We will review the evidence for each of these cells in turn, although it is likely that there is movement of cells between compartments and many resident or

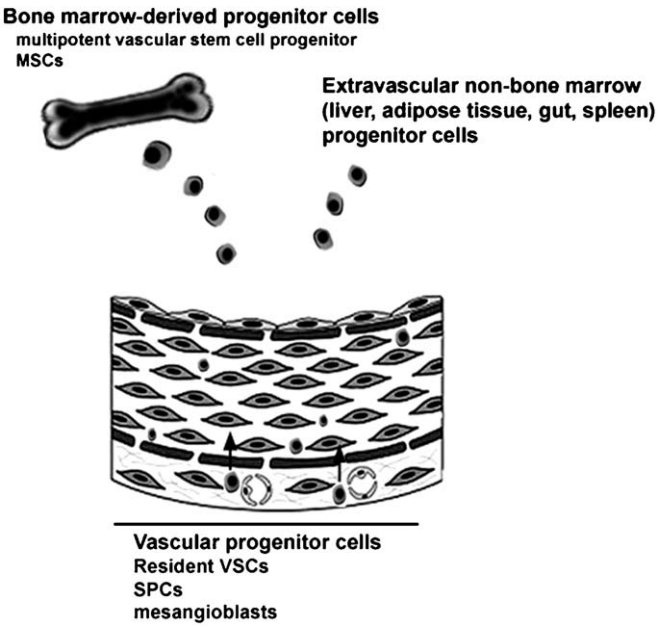

Fig. 2. Schema of possible sources of vessel-derived and extravascular SMCs. A full description is provided in the text.

extravascular progenitor cells may ultimately be derived from the bone marrow by way of the circulation. Identification of these cells has frequently required characterization and expression of specific stem or progenitor cell antigens that have been also identified in a population of vascular smooth muscle cells in the normal vessel wall or disease. These expression markers are listed in Table 1, together with the cell types currently identified that express them.

3.1. Multipotent vascular stem cell progenitors

Hemangioblasts, a multipotent progenitor cell capable of simultaneously developing hematopoietic and endothelial lineages, have been isolated within blood islands in the developing mouse embryo [37]. By using mouse and human embryonic stem cell cultures, blast-colony-forming cells were isolated and expanded clonally to produce both endothelial and hematopoietic cells when stimulated with vascular endothelial growth factor-A (VEGF-A) and bone morphogenetic protein-4 [38,39]. Although hemangioblasts are believed to derive from mesodermal cells, recent studies suggest that the hemangioblast does not give rise to all endothelial and hematopoietic lineages, at least in mouse and zebrafish embryos [37]. There is also increasing evidence to support the presence of hemangioblasts in adult organisms, since multipotent mesenchymal stem cells have been isolated from

Table 1
Progenitor or stem-derived surface and cytoplasmic antigens in VSMC progenitors and VSMCs.

Antigen	Origin	Cell type
CD133	Transmembrane glycoprotein	Hemangioblasts, SPCs
CD34	Transmembrane sialomucin protein	SPCs, hematopoietic stem cells
Flt-1 (VEGFR-1)	Transmembrane kinase receptor	SPCs, neointimal VSMCs
KDR (VEGFR-2)	Transmembrane kinase receptor	SPCs, neointimal VSMCs
c-kit (CD117)	Membrane receptor for stem cell factor	Hemangioblasts, neointimal VSMCs
CD45	Protein tyrosine phosphatase	SPCs, hematopoietic stem cells
CD14	Cytoplasmic endotoxin receptor	SPCs
sca-1	Transmembrane class III tyrosine kinase receptor	Hematopoietic stem cells, adventitial progenitor cells
Notch-1	Transmembrane regulatory receptor	Side population-derived VSMCs, neointimal VSMCs
Stro-1	Trypsin-resistant cell surface antigen	MSCs-derived VSMCs
SSEA1	Cell membrane antigen	MSCs-derived VSMCs
CD105	Cell membrane antigen	MSCs

SPC = smooth muscle progenitor cell; sca-1 = stem cell antigen-1; MSC = mesenchymal stem cell.

adult mouse and human bone marrow that are capable of producing mature endothelial cells *in vitro* and *in vivo* [40,41]. Recently, hemangioblasts from human embryonic stem cells have been shown to generate multilayered blood vessels with functional smooth muscle cells *in vitro* [42] opening new prospects for the use of hemangioblasts as a potentially inexhaustible source of cells for the treatment of human blood and vascular disease.

3.2. Mesenchymal stem cells

Within bone marrow fractions, mesenchymal stem cells (MSCs) are defined as 'non-haemopoietic stromal cells that are capable of differentiating into mesenchymal tissues, such as bone, cartilage, muscle and adipose' and can home to the site of injury after both site-directed and systemic administration [43]. MSCs also possess the greatest ability to differentiate into cells expressing SMC markers in culture and the highest incorporation into the neointima [44,45]. *In vitro*, human bone marrow-derived MSCs cultured in the presence of laminin, collagen type IV and fibronectin proliferate and express smooth muscle-specific genes, including alpha smooth muscle actin (α-SMA) and calponin, and these markers are retained when these cells are implanted *in vivo* [46]. MSCs can also be derived from non-bone marrow sources. For example, adipose-derived stem cells isolated from human lipoaspirate represent a reliable source of MSCs with multi-differentiation potential and differentiate into α-SMA and smooth muscle myosin heavy chain (SMMHC)-positive SMCs after administration of transforming growth factor-β1 (TGF-β1) and bone morphogenetic protein-4 [47]. These findings suggest that growth factors and cell-matrix interactions may represent signals for the differentiation of MSCs into VSMCs within the vessel wall and MSCs again may represent a reservoir for cardiovascular tissue engineering.

3.3. Circulating vascular smooth muscle progenitor cells

Vascular progenitor cells that can differentiate in culture into either endothelial or smooth muscle lineages have been isolated from peripheral human blood [48,49]. The smooth muscle progenitor cells have been identified by the expression of markers of mesenchymal/smooth muscle lineage when they differentiate, such as SMMHC, endoglin and calponin and share with endothelial progenitor cells an endoglin $^{+}$/CD14^{+} phenotype [49]. Studies using parabiosis models have also shown that endogenous circulating cells can contribute to neointima formation without the added complexities of irradiation and bone marrow transplantation [50]. However, human blood-derived smooth muscle progenitor cells exhibit an immature, highly proliferative and pro-inflammatory phenotype, with higher levels of mRNA encoding IL-18, IFN-γ, iNOS, p22phox and MMP-9 than aortic normal media-derived SMCs [48] and in part resemble the dedifferentiated SMC phenotype observed in atherosclerotic plaques and the neointima (see above). Cultured CD34^{+}-enriched cells from human umbilical cord blood samples cultured on type I collagen in a growth factor-enriched medium can also give rise to endothelial progenitor cells (characterized from expression of endothelial markers endothelial markers including Factor VIII, CD31) and smooth muscle progenitor cells (negative for endothelial markers but positive for specific myocytic marker calponin) [51].

3.4. Resident smooth muscle progenitor cells in adult vessels

As mentioned above, VSMC accumulation in the fibrous cap of an atherosclerotic plaque is monoclonal or oligoclonal [6,7]. The original explanation of this phenomenon was that a small number of medial VSMCs undergo proliferation in response to the injurious stimuli associated with atherosclerosis. Subsequent studies examining telomere loss indicate that fibrous cap VSMCs have undergone 10–14 more population doublings than cells in the normal media [8], suggesting the existence of a resident arterial subpopulation that contributes to arterial healing in response to injury [9]. Whilst this could mean a mutation leading to cell transformation, no consistent cellular oncogene has been found to be overexpressed in plaques and subsequent studies indicated that the normal vessel wall comprises 'patches' of VSMCs with one marker, so that plaques arise by selective expansion of a pre-existing 'patch' of progenitor cells. Indeed, in bovine and canine vessels, subsets of medial VSMCs have been demonstrated that have robust proliferation and autonomous growth with down-regulated VSMC markers, reminiscent of the fetal SMC phenotype [52,53].

Most of the studies aimed at characterizing a population of resident proliferative cells in the normal arterial media have been performed in the arterial wall of rodents. A population of resident proliferative cells has been identified in the normal arterial media in healthy adult mice, varying from 6 to 15% in the aorta or carotid artery [54]. These tunica media-derived resident progenitor cells were sca1^{+}, c-kit-low and CD34-low, and underwent endothelial differentiation when treated with VEGF and SMC differentiation in the presence of TGF-β1 or PDGF-BB. These workers also found that progenitor cells with the same profile were absent in the adventitia [54]. These progenitor cells are different from marrow-derived smooth muscle progenitor cells identified in skeletal muscle, since they lack the ability to differentiate into erythroid, lymphoid, or myeloid tissue [55]. A subpopulation of vascular cells has been also identified derived by dilutional cloning of bovine aortic medial VSMCs that are capable of producing multiple lineages similar to MSCs [56]. These cells were CD29^{+} and CD44^{+} but CD14^{-} and CD45^{-}, consistent with other MSCs and retained multipotentiality despite passaging through more than 20–25 population triplings, indicating a capacity for self-renewal. The adult rat aortic media also contains some progenitor cells with pericyte-like properties, including anchorage-independent cells that proliferate slowly in suspension, forming spheroidal colonies [57]. In the presence of serum, cells from spheroid colonies become CD34^{-} and express SMC markers [57].

In addition to the tunica media, progenitor cells that can form mature VSMCs have been identified in the adventitial layer of adult mouse aortas [58]. These cells express Sca-1 and differentiate into VSMCs when exposed to PDGF-BB and into endothelial cells when exposed to VEGF-A *in vitro*. Although the role of these cells in native atherosclerosis is unclear, they appear to contribute to graft neointimal formation when transplanted into the adventitia of vein grafts [58]. Satellite-like cells named 'mesoangioblasts' can also be isolated from explants of murine dorsal aorta and are able to differentiate into skeletal muscle, smooth muscle and other mesenchymal cell types *in vitro* [59]. Mesoangioblasts express both myogenic and endothelial cell markers (particularly Flk1–VEGF receptor), and are phenotypically distinct from multipotent hemangioblasts [60], which express and can give rise to both hematopoietic and endothelial progenies [59]. Mesoangioblasts, when incorporated into grafted host blood vessels, are successively dispersed by the circulation and appear integrated into a wide range of mesodermal-derived tissues, including cartilage, bone, smooth muscle and cardiac muscle [61]. Gene expression profiles of mesoangioblasts determined by DNA micro-array analysis reveals that mesoangioblasts express genes belonging to response pathways activated by developmental signaling molecules, such as Wnt or TGF-β1/bone morphogenetic protein [62]. Interestingly, mesoangioblasts express receptors of the TGFbeta/BMP family and several Smads and, accordingly, differentiate very efficiently into VSMCs in response to TGF-β1 [62].

In contrast to rodents, there is very limited evidence for the presence of vessel wall stem cells in human vessels. A population of $CD34^+$, $CD31^-$ cells has been identified in the space between the media and adventitia of large and medium-sized human arteries and veins [63,64]. Unlike the subpopulation reported in mice [54,58], these cells could contribute to newly formed capillary-like tubes *ex vivo*, but their capacity to give rise to VSMCs was low [63]. Cultured human vascular adventitial fibroblasts from pulmonary arteries were also found to contain a progenitor cell-like population ($CD29^+$, $CD44^+$, $CD105^+$), negative for hematopoietic and endothelial cell markers [65]. These cultured human vascular adventitial fibroblasts did not display the typical 'hill-and-valley' growth pattern of normal medial VSMCs *in vitro,* but could undergo osteogenic, adipogenic and myogenic differentiation, the latter characterized by increased expression of α-SMA and calponin [65].

3.5. Extravascular, non-bone marrow origin of smooth muscle cells

Multipotential cells capable of differentiating into VSMCs have been obtained diverse sites of adult human non-vascular and non-bone marrow tissue, including adipose tissue [66]; amniotic fluid [67] and lymphoid follicular dendritic cells [68]. Whilst the vasculogenetic properties of these cells vary, leading to the development of capillaries to more complex and specialized arterial structures, the mechanisms regulating mobilization, differentiation and integration of these potential vascular progenitor cells in normal vessels, new vessels and vascular disease remain almost unproven. Recently, $VEGFR\text{-}2^+$/$c\text{-}kit^+$ resident coronary vascular progenitor cells have been isolated from human myocardial samples. These myocardial vascular progenitor cells were self-renewing and clonogenic and differentiated predominantly into endothelial cells and VSMCs and partly into cardiomyocytes. Moreover, myocardial vascular progenitor cells were capable of generating competent coronary vessel VSMCs and to contribute positively to coronary blood flow after induction of a vascular stenosis[69].

In summary, there is evidence for several distinct resident progenitor cells in different layers of the normal adult arterial wall capable of proliferating and differentiating into VSMCs. What has not yet been established is whether and by how much these cells contribute to formation of vascular lesions and whether clonality reflects selective proliferation of one or more of these populations. In addition, whilst it is clear that both bone marrow and non-marrow-derived progenitors can contribute to VSMCs in vascular lesions, their true contribution and role has not yet been determined.

4. Regulation of differentiation of progenitor cells into VSMCs

Whilst the signaling pathways that regulate VSMC differentiation in embryogenesis and disease are well documented (reviewed in [70]), it is unclear whether similar pathways are responsible for differentiation of bone marrow-derived or vessel wall-derived progenitor cells into VSMCs in the vessel wall. However, there is likely to be a considerable overlap. For example, Hedgehog-induced Notch-1 expression increases in intimal VSMCs of murine carotid arteries after vascular injury [71] and Notch-1 expression is involved in phenotypic modulation of VSMCs during development, in particular in controlling VSMC differentiation from neural crest cells [72]. Jagged-1-induced Notch signaling also promotes VSMC differentiation [73]. Over-expression of Notch I up-regulates the expression of multiple VSMC marker genes, including SMMHC [73] and Notch activation also results in morphological, phenotypic and functional changes consistent with endothelial-to-mesenchymal transformation, with down-regulation of endothelial markers vascular endothelial (VE)-cadherin, Tie1, Tie2, platelet-endothelial cell adhesion molecule-1 and endothelial NO synthase and up-regulation of mesenchymal markers α-SMA, fibronectin and PDGF receptors [74,75]. Similarly, agents that promote expression of smooth muscle cell transcription factors (e.g. myocardin) such as thrombin can promote the differentiation of bone marrow-derived cells into VSMCs [76], although myocardin is not required for smooth muscle cell differentiation from embryonic stem cells [77].

5. Frequency of progenitor cell-derived VSMCs in vascular disease

Until quite recently, the vessel wall was viewed as the sole source of VSMCs in atherosclerosis and neointima formation after injury of transplant disease [78]. The discovery that progenitor cells contribute to endothelial repair and VSMC accumulation has challenged this classical pathogenetic model of vascular lesions. However, there is still huge controversy over both the frequency and the role of extravascular progenitor cell-derived VSMCs in vascular disease, predominantly because of the difficulty of tracking these cells *in vivo* and the presence of monocytic inflammatory cells in many vascular diseases. The identification of VSMC progenitors has been performed mostly by tracking using either a bone marrow or progenitor cell marker, co-localized with VSMC markers *in vivo*.

5.1. Tracking by bone marrow markers

Over the last decade, there have been multiple studies reporting that other sources of cells contribute to the development of atherosclerotic, neointimal and transplant atherosclerotic processes, predominantly from the bone marrow or circulating cells [10,11,48,79–83]. In most of these studies, a bone marrow or circulating cell marker has been found to co-localize with one or more VSMC markers in a variety of vascular lesions, including atherosclerosis and after injury. For example, studies employing bone marrow transplantation in mice revealed that bone marrow-derived cells marked by green fluorescent protein contribute up to 60% of VSMCs in the neointima after arterial injury [11]. Donor-derived cells (marked by Y chromosome) also comprised up to 10% of VSMCs within atherosclerotic lesions in humans receiving sex-mismatched bone marrow transplantation [12,13].

As mentioned above, these studies are mainly based on the morphological evidence of co-expression of stem antigens or recognizable sex-mismatched cells and smooth muscle markers. There are very significant technical problems with reliance on this methodology for estimation of frequency of marrow-derived VSMCs in lesions. For example, precise co-localisation of two markers to a single cell in a complex tissue requires very high quality preparation of the material and Z-series reconstruction using confocal microscopy, techniques that have been rarely performed. In addition, many studies rely solely on α-SMA as a VSMC marker; α-SMA is known to be expressed outside of VSMCs and all VSMC markers are down-regulated in vascular disease [70].

For these reasons, other studies have failed to reproduce the initially very high percentage of extravascular-derived VSMCs in disease. For example, in transplant arteriopathy, neointimal VSMCs appeared to originate mainly from the recipients and not from the donor bone marrow-derived cells [10]. Similarly, using double fluorescence microscopy and Z-series reconstruction Bentzon et al. found no evidence of bone marrow origin of VSMCs in mice [14], even after plaque disruption and VSMC recruitment for repair [15]. These workers concluded that VSMCs in advanced atherosclerotic lesions and healing after plaque rupture originate from the vessel wall and not from bone marrow or circulating progenitor SMCs.

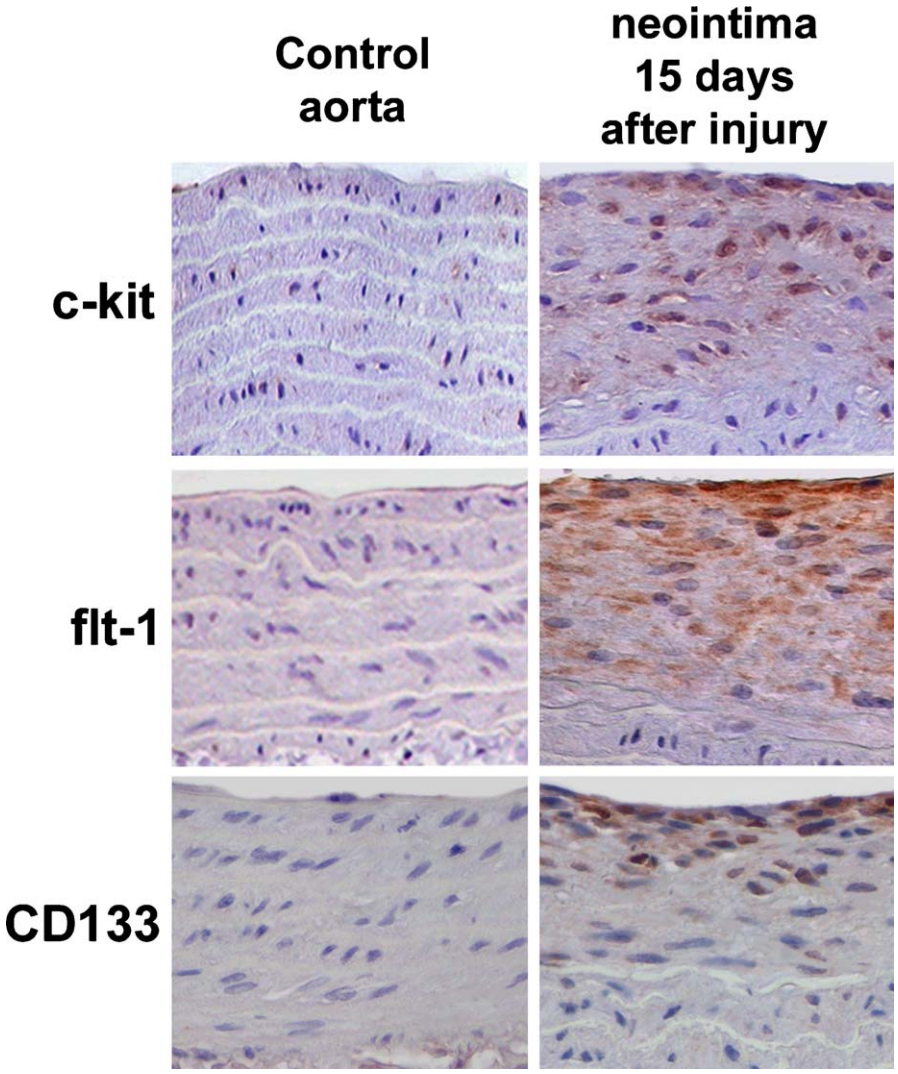

Fig. 3. Stem cell marker expression in rat aortic tissue.Formalin-fixed sections of normal rat aorta (left column) and neointima and underlying tunica media fifteen days after endothelial injury by ballooning (right column) stained with monoclonal anti-flt-1, anti-c-kit and anti-CD133 antibodies using diaminobenzidine as a chromogen. Original magnification, 200×.

5.2. Tracking by stem cell markers

An alternative method of identifying stem or progenitor cell-derived VSMCs in vascular lesions is by co-localisation with stem or progenitor cell markers. For example, MSCs express a variable combination of the markers CD133, CD44, Stro-1, SSEA1 and CD105 [40]. Hemopoietic stem cells express a variety of markers, including stem cell antigen-1 (sca-1) and the tyrosine kinase receptor (c-kit). The early functional angioblasts also express *fms*-like tyrosine kinase (flt-1). c-kit/CD117 and CD133 are variably documented in human atherosclerotic plaques and restenosis lesions [16,84] and in post-injury rat aortic intimal thickening [85]. For example, human in-stent and angioplasty restenosis tissue specimens contained cells that immunolabeled for c-kit and SMA, whereas the intima and media of primary atherosclerotic plaques and normal arteries were devoid of c-kit-immunopositive cells [84]. Similarly, Torsney et al. demonstrated a small number (<1%) of progenitor cells expressing sca-1 and c-kit/CD117 within human plaques and the adventitia, with variable expression of CD34, sca-1, c-kit and VEGF receptor 2 (VEGFR2) markers, but no CD133 expression [16]. Progenitor cell markers are also expressed in vessels after injury in animal models. For example, whereas rare flt-1$^+$ and c-kit$^+$ VSMCs are detected in normal rat aorta, their number increases fifteen days after injury, so that intimal cells express both α-SMA and flt-1 (61.8 ± 3.8%), c-kit (45.7 ± 3%) or CD113 (5.6 ± 0.7%) [17]. After 60 days, intimal stem marker-expressing cells had almost disappeared whereas α-SMA expression was restored (Fig. 3).

6. Role of progenitor cell-derived VSMCs in vascular disease

The majority of studies have identified the presence and frequency of progenitor-cell-derived VSMCs in vascular disease. However, very few studies have identified the role these cells play in disease. As mentioned above, human blood-derived smooth muscle progenitor cells exhibit an immature, highly proliferative and pro-inflammatory phenotype [48]; mobilisation of these cells would therefore be predicted to promote vascular disease. Indeed, enhanced progenitor cell recruitment and increased neointima formation have been seen after Granulopoiesis-stimulating factor (GSF) treatment in some animal models after stenting [86] and a trend to increased restenosis was also seen after GSF treatment in humans after myocardial infarction and stenting in one study [87], although recent meta analysis of several small studies was reassuring [88].

In contrast, injection of smooth muscle progenitor cells reduced the progression of early (but not more advanced) atherosclerotic plaques in a mouse model of advanced atherosclerosis, suggesting that a recruitment of these smooth muscle progenitor cells promotes plaque stabilization [51]. Although this model used injection of human progenitors into a complex nude mouse atherosclerosis model, patients with acute coronary syndrome show reduced peripheral blood-derived progenitor cells expressing smooth muscle markers compared those with stable angina patients [51]; the latter have lower levels of putative smooth muscle progenitor cells in circulating blood, similar to healthy subjects [48]. These findings suggest that circulating smooth muscle progenitor cells may play a beneficial role in suppressing atherosclerosis or its consequences. However, detailed studies demonstrating whether VSMC progenitors either protect or promote vessel disease are needed before cell-based or pharmacological approaches aimed at regulating progenitor cell trafficking can be recommended.

7. Conclusions

Vascular smooth muscle cell progenitor cells may contribute to both the homeostasis of the normal arterial wall and to vascular disease. There is some evidence to suggest that progenitor cells are both resident in the normal arterial wall and derived from a variety of extravascular sources, including the bone marrow and circulating cells. Progenitor cell mobilisation occurs in atherosclerosis, after arterial injury or in allograft disease and can be tracked using genetic markers or co-localisation of stem and VSMC markers. However, the presence and extent of VSMC progenitors in lesions depends upon the model, time and species studied and the role of these cells is unclear. Whilst the selective control of VSMC progenitors in the arterial wall may represent an attractive therapeutic possibility, more detailed studies to define both their frequency and their effects on vessel disease are required.

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