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Progenitor Cells in Arteriosclerosis: Good or Bad Guys?

Paola Campagnolo, Mei Mei Wong, and Qingbo Xu

Abstract

Accumulating evidence indicates that the mobilization and recruitment of circulating or tissue-resident progenitor cells that give rise to endothelial cells (ECs) and smooth muscle cells (SMCs) can participate in atherosclerosis, neointima hyperplasia after arterial injury, and transplant arteriosclerosis. It is believed that endothelial progenitor cells do exist and can repair and rejuvenate the arteries under physiologic conditions; however, they may also contribute to lesion formation by influencing plaque stability in advanced atherosclerotic plaque under specific pathologic conditions. At the same time, smooth muscle progenitors, despite their capacity to expedite lesion formation during restenosis, may serve to promote atherosclerotic plaque stabilization by producing extracellular matrix proteins. This profound evidence provides support to the hypothesis that both endothelial and smooth muscle progenitors may act as a double-edged sword in the pathogenesis of arteriosclerosis. Therefore, the understanding of the regulatory networks that control endothelial and smooth muscle progenitor differentiation is undoubtedly fundamental both for basic research and for improving current therapeutic avenues for atherosclerosis. We update the progress in progenitor cell study related to the development of arteriosclerosis, focusing specifically on the role of progenitor cells in lesion formation and discuss the controversial issues that regard the origins, frequency, and impact of the progenitors in the disease. *Antioxid. Redox Signal.* 15, 1013–1027.

Introduction

BLOOD VESSELS are composed mainly of two types of cells, endothelial cells (ECs) that line the lumen and smooth muscle cells (SMCs) that form the structure of the media. The vascular system is the first functional organ to develop in the vertebrate embryo. This process is initiated by the formation of the blood islands (21). ECs develop from these islands, with mesenchymal cells from the splanchnic mesoderm differentiating to contribute to the EC population (144). The new vessel develops from this primitive tube plexus. The following phase is the acquisition of a tunica media for the larger vessels, such as arteries and veins. At first, endothelial tubes are enveloped by mural cells to form SMCs and pericytes, as seen during nascent vascular and cardiac valve development (2). Conversely, SMCs have a complex and heterogenic origin during embryo development (59). Thus, both cell types are derived from progenitor cells that are crucial for the formation of new blood vessels.

In adults, large and middle-sized arteries undergo remodelling in response to the fluctuation of blood pressure (145), whereas small vessels and microvessels can be regenerated in damaged tissues through angiogenesis or vasculogenesis (79, 80). In these processes, both ECs and SMCs are actively involved. Emerging data provide strong postulations of the existence of a population of vascular progenitor cells in a variety of tissues. Intriguingly, these cells are capable of differentiation into ECs and SMCs, thereby participating in angiogenesis and vascular remodelling (1, 4, 52, 131, 148). In 1997, endothelial progenitor cells (EPCs) were isolated primarily as $CD34^+$ hematopoietic progenitors from peripheral blood (7). These cells represented a heterogeneous population that expressed CD34 or VEGFR2 markers, which share common properties and functions and can proliferate and differentiate into ECs *in vitro*. More recently, Simper *et al*. (123) reported the identification of a population of vascular smooth muscle progenitor cells (SPCs) in circulating blood. Subsequently, several articles demonstrated the presence of vascular progenitors in other tissues [*e.g*., adventitia (58) and bone marrow (4)]. Since then, a large number of reports (>2,000) related to EPCs and SPCs have been published. The majority of data produced focus largely on several key issues: the mobilization and specific localization of progenitor cells and their functional roles in both physiologic and pathologic conditions of atherosclerosis (148, 149, 158).

Arteriosclerosis is characterized by SMC hyperplasia or hypertrophy and matrix protein accumulation in the intima or media or both, with or without lipid deposition, resulting in

Cardiovascular Division, King's College London BHF Centre, London, England.

thickening and stiffness of the arterial wall (125). Arteriosclerosis includes spontaneous atherosclerosis, accelerated (transplant) arteriosclerosis, vein-graft atherosclerosis, and restenosis after percutaneous transluminal coronary angioplasty (147). Atherosclerosis is a progressive disease characterized by the formation of atheromatous plaques within the walls of large and medium-sized arteries. Early lesions, otherwise known as fatty streaks, may occur in the intima as early as childhood and develop into plaques with a lipid-rich core within the central portion of the thickened intima in adults. The fibrous cap may also rupture at later stages, and the release of lipids results in a signal cascade that leads to thrombus formation (107), thereby contributing to arterial occlusions, coronary disease, myocardial infarction, and stroke. Endothelium turnover and the proliferation of SMCs are important events in the pathogenesis of atherosclerosis (106, 108, 109). Traditionally, it is believed that during atherosclerotic plaque or neointima formation or both, neighboring cells, such as mature ECs and SMCs from the media, migrate to the intima to replace dead ECs through replication and assumption of synthetic phenotypes. However, recent data strongly suggest that new sources of ECs and SMCs differentiated from EPCs and SPCs recruited from adventitia, resident vascular stem/progenitor cell niche, the circulation, and other sites may also participate in atherosclerotic plaque development and neointima formation (29, 35, 48, 58, 114, 136, 157). We provide further insights into the impact of vascular progenitors in the development of arteriosclerosis, specifically focusing on the nature, characterization, mechanisms of differentiation, and controversial issues regarding progenitor cells in the disease.

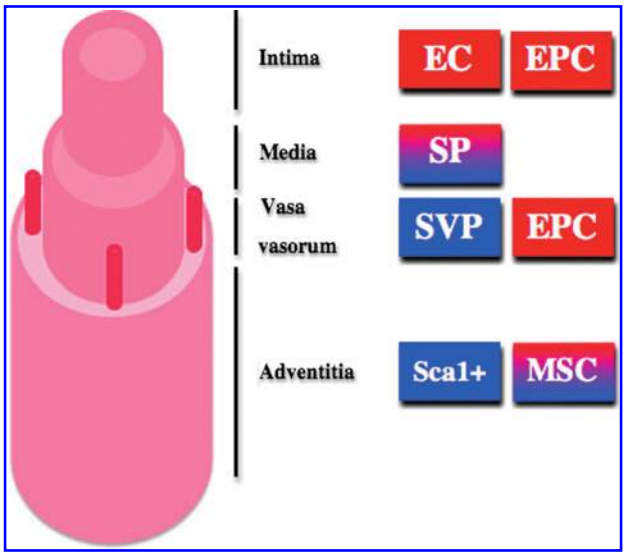

FIG. 1. Scheme illustrating the different progenitors populations in the vessel wall. Several populations were isolated from each layer of the vessel: the intima layer includes endothelial cells (ECs) capable of clonal expansion; a side population (SP) of ABGC2 cells was isolated from the media; in the adventitia reside vasa vasorum pericytes-like (SVP) and endothelial progenitor cells (EPC); and in the adventitia, $Sca1^+$ and mesenchymal stem cells (MSCs) were found. Color code indicates the plasticity of the populations toward ECs (red) or mural (blue) lineages (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Blood Vessel-Resident Progenitor Cells

Adult vessels are composed of rather quiescent cells that can be activated as a result of endothelial injury and atherosclerosis (107). Given this plasticity, it is not surprising that adult blood vessels have been shown to harbor several different progenitor populations (66) (Fig. 1). In 2004, Hu *et al.* (58) demonstrated the presence of a resident population of mesenchymal Sca-1^+ cells isolated from the aortic adventitia of Apo-E knockout mice, which may act as a source of SMCs during neointima formation. These elements might contribute to media homeostasis and to the replenishment of the pool of circulating SPCs. These findings also supported the hypothesis that Sca-1^+ cells in the adventitia can contribute to the accumulation of SMCs in atherosclerotic lesions largely *via* direct migration across the media or through circulating blood (58). Studies have shown that the tunica media of adult rat artery contains a population of putative progenitor cells that were largely CD34 and Sca-1 positive. Furthermore, the progenitor cells exhibited high expression of the membrane transporter ABCG2. Functional expression of this transporter, enabling cells to exclude the fluorescent dye Hoechst 33342, is a typical feature of a so-called "side population" of progenitor cells (111). Furthermore, the progenitors were able to form vascular structures by differentiating into both VE-cadherin–positive ECs and α-SMA–positive smooth muscle cells under three-dimensional (3D) matrix culture conditions (111).

Concomitantly, several groups focused on the characterization of mesenchymal cells from the vessel wall. Both human saphenous vein and thoracic aorta were shown to harbor a population of cells positive for mesenchymal markers (including CD13, CD29, CD90, CD44, and CD54) that were able to give rise to all mesenchymal lineages (8, 9). In particular, aorta-derived progenitors also were shown to possess angiogenic characteristics *in vitro* by using Matrigel assays (9). Recently, Covas and co-workers (23) demonstrated that perivascular stem cells are harbored in most human tissues and can be isolated by using the marker CD146 (23). This is consistent with the results of other groups showing that MSCs and perivascular cells (pericytes) share functional properties and antigenic profiles (88). Covas *et al.* (23) compared a large number of fibroblasts, progenitors, and pericyte cell lines from both adult and fetal tissues (including umbilical cord vein and artery, fetal artery, and adult vena saphena) and established that all the studied cell lines were homogeneously expressing mesenchymal markers and differentiating into osteblasts, chondrocytes, and adipocytes. This evidence provides support for the hypothesis that pericytes are a population of residing progenitor cells in peripheral organs (Fig. 1). The vasa vasorum of the vena saphena was indeed demonstrated to harbor $CD34^+$ cells with pericyte characteristics that possess proangiogenic activity (saphenous vein progenitor cells) (18).

Because ECs play a fundamental role in atherosclerosis by releasing oxidative species and leading the interaction with SMCs and macrophages (40), a deeper understanding of the existence of EPCs in the vessel wall and their potential contributions to atherosclerosis is therefore of the utmost importance. Primarily, it must be noted that recent publications challenged the very same definition of mature vascular ECs as

a population of terminally differentiated elements. An elegant series of experiments demonstrated that luminal ECs contain a hierarchy of cells at different stages of differentiation, a few of them being clonogenic and proliferating progenitors (60). EPCs from the human internal thoracic artery were isolated by using a well-established assay with an *ex vivo* culture of vessel rings in 3D biomatrices. Neovessels formed in this model are composed of a luminal layer of ECs and surrounding pericytes. The origin of these neovessels was seemingly attributable to differentiated cells from the vessel wall. Conversely, studies on human artery specimens showed that a population of immature progenitors might instead contribute to the phenomenon (161). Indeed, $CD34^+$ KDR^+ EPCs residing in the artery wall were able to form capillary sprouts in the artery ring assay. Furthermore, the cells were found to acquire endothelial markers and cell-adhesion molecules, such as VEGF receptors, Tie2, and VE-cadherin, thereby indicating their commitment into the endothelial lineage (161). The authors also identified a CD133-positive population in the matrix in which the rings were embedded; and although they did not investigate further on the function of those cells, they hypothesized that the $CD133^+$ cells may act as precursors of macrophages. Therefore, further investigation of the contribution of this population to inflammation and atherosclerosis is needed.

Bone Marrow–Derived Vascular Progenitors

The bone marrow is the major reservoir of adult stem cells, fulfilling the constant need for new cells in the body (4). Stimuli (*e.g.*, VEGF produced by vascular injury or by tumor growth) determine the release of pro-angiogenic cells from the bone marrow into the circulation, where they migrate and home to the damaged tissue (114) (Fig. 2). Of interest, bone marrow can release progenitors that serve as EPCs (4). Despite an abundance of published data, the precise cell-surface marker and contribution of circulating EPCs to angiogenesis remains a controversy. This is related to the fact that a variety of subsets from circulating cells have similarly been shown to have the capacity to acquire the endothelial phenotype (7, 32, 46, 97). To date, the most widely used markers for identifying putative circulating EPCs are either $CD133^+CD34^+VEGFR2^+$ or $CD34^+VEGFR2^+$ (7, 97, 132), although the cells have also been described as $CD34^+$, along with the co-expression of hematopoietic antigens such as CD45. After *in vitro* propagation, EPCs stop expressing CD45 (leukocyte common antigen) and acquire EC markers, such as factor VIII, CD31, UEA-1 (*Ulex eureopaeus* agglutinin-1), eNOS (endothelial nitric oxide), and E-selectin, and become able to incorporate Dil-labeled acLDL.

Seminal studies showed that injected EPCs were able to integrate into nascent capillaries of limb ischemic non-immunocompetent mice. After these early studies, EPCs were later shown to be incorporated into nascent vasculatures of pathologic lesions, tumor and wound-healing sites, as well as during endometrium formation (6). However, recent data provide evidence of existing myeloid subpopulations (*i.e.*, $CD14^+CD34low$, $CD14^+VEGFR2^+CXCR2^{+/-}$, and $CD14low$ $CD16^+Tie\text{-}2^+$ cells that also displayed angiogenic characteristics, therefore challenging the initial characterization of EPCs (32, 53, 133)]. Classically, EPCs are cultured by plating blood mononuclear cell fraction on fibronectin-coated dishes

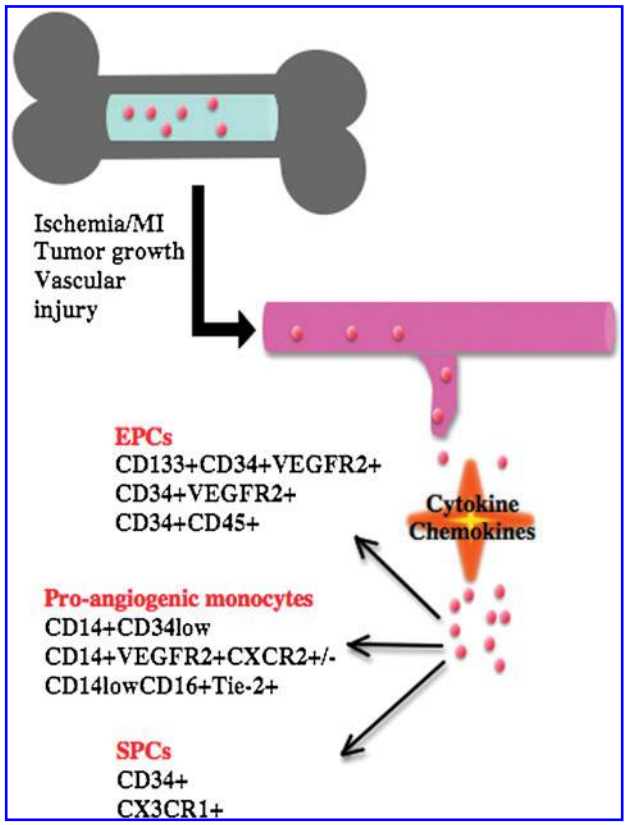

FIG. 2. Bone marrow as a source of vascular progenitor cells. Pathologic stimuli such as ischemia, tumor, or vascular injury mobilize progenitor cells from the bone marrow and attract through the blood flow by release of chemoattractants. Once *in situ*, bone marrow-derived progenitors undergo differentiation depending on the local microenvironment (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

in endothelium-specific growth medium, thereby giving rise to cells with two distinct phenotypes, the first being adherent spindle-shaped cells that develop after 4 to 7 days of culture and have been described as endothelium-like or "early" EPCs (53, 62, 103, 105). These cells express markers typical of both monocytes and ECs, secrete angiogenic cytokines, but possess only low angiogenic capacity (53, 62, 105). The second phenotypically distinct cell subset develops after 3 to 4 weeks in culture, whereby a "late" proliferative outgrowth arises. These cells show characteristics of mature ECs and possess the ability to form functional vessels and to attenuate neointimal hyperplasia *in vivo* (43, 75, 134, 156). Several studies have also demonstrated the therapeutic potential of late EPC transplantation in mouse and rabbit models of hindlimb ischemia (63, 127). *Ex vivo* expanded EPCs reportedly incorporate into foci of myocardial neovascularization (64). Furthermore, intracoronary infusion of mononuclear cells in patients with acute myocardial infarction was shown to expedite postinfarction remodeling (17, 65, 85). Nevertheless, despite the plethora of evidence to support the proangiogenic capacity of EPCs, their direct incorporation into the endothelium to repair damaged vessels still remains to be elucidated.

Furthermore, the reported rate of EPC incorporation into the growing vasculature varies from 0 to 90%, and quantification is potentially biased because of the difficulty in distinguishing real luminal ECs from infiltrating macrophages and monocytes by using existing microscopic techniques (129).

In addition to EPCs, SPCs have been isolated from human peripheral blood. Analysis of mononuclear cells cultured in presence of PDGF-BB showed positivity for SMC markers (α-SMA, smooth muscle myosin heavy chain, and calponin), as well as CD34 and the VEGF receptors (VEGRR1 and VEGFR2). Furthermore, blood cell–derived $CD34^+$ cells could also be induced *ex vivo* to differentiate into both SMCs and ECs (72). More recently, a small population of $CX3CR1^+$ cells in the bone marrow were shown to be able to generate mature and functional SMCs in culture (89) and to home to sites of vascular injury to constitute about 5% to 10% of the neointima (68). These data demonstrate that the bone marrow is an abundant source of vascular progenitor cells.

Vascular Progenitors Derived from Other Tissues

Besides the bone marrow and vascular tissue, several other organs have been shown to harbor progenitor cells that give rise to ECs and SMCs. For instance, both murine and human $Isl1^+$ cells (cardiomyocyte precursors) from embryonic hearts were capable of differentiation into ECs and SMCs (48, 49). Furthermore, c-kit^+ cells isolated from human hearts were shown to be able to differentiate into cardiomyocytes, myogenic, smooth muscle, and endothelial cell lineages when transplanted into infarcted myocardium, although structural organization and complete morphologic differentiation were not achieved (9). More recently, multipotent progenitor cells, clonally expandable and able to give rise to cells from all the three germ layers (including ECs), were generated from human heart and liver (10). Human liver–derived EPCs that were isolated according to their $VEGFR2^+$ $CD146^+$ $CD45^-$ expression by using flow cytometry were capable of induction into mature endothelial cells (99). Similarly, mouse spleen-derived endothelial progenitor cells were able to reduce neointima formation when injected systemically after carotid artery injury (138).

An interesting new source of autologous progenitor cells is the adipose tissue (92, 94). This tissue provides an easily accessible source of progenitor cells; large number of cells can be obtained through a minimally invasive harvesting procedure (*i.e.*, liposuction). Besides that, the stromal vascular fraction is also known to contain different populations of progenitors that are able to promote reparative angiogenesis in a model of limb ischemia (92, 94). It has been proposed that adipose tissue–derived $CD34^+$ cells act as resident pericytes, expressing typical markers such as NG2 and PDGFRβ. The adipose tissue pericytes were shown to promote the survival of ECs through the release of various angiogenic factors and were also able to incorporate into EC-formed tubelike structures on Matrigel (130). Taken together, this evidence provides support for the hypothesis that the progenitors resident in different tissues might be directly or indirectly involved in repairing or replacing damaged ECs and SMCs.

Endothelial Repair by Progenitor Cells: Good Guys?

Atherosclerosis is a consequence of a complex sequence of events in which EC dysfunction/death is an initial event. Evidence exists of structural and functional heterogeneities in the endothelium of large and middle-sized arteries (39, 116). Foteinos *et al.* (35) found that the rate of EC turnover is largely associated with the pattern of blood flow in atherosclerotic apoE-deficient mice, which is strictly related to the development of atherosclerosis (35). Based on data from animal models, ECs in the areas resistant to atherosclerosis have a lifespan about 12 months, whereas cells at lesion-prone sites survive for only several weeks, or even less in the case of aged animals (118). The mechanisms of athero-prone area–related EC death involve signal pathways leading to EC apoptosis in the presence of hyperlipidemia, initiated primarily by endoplasmic reticulum stress. Supporting this notion is the fact that overexpression of endoplasmic reticulum stress-induced X-box–binding protein 1, a key signal transducer of the endoplasmic reticulum stress response, *in vivo* and *in vitro* were highly increased in atherosclerosis-prone areas in vessel walls (160). The development of atherosclerosis is a consequence of damaged ECs not being properly repaired or replaced (Fig. 3). A regenerative response occurs in the intima after EC death, which involves two types of cells, mature ECs and EPCs present in the vessel wall and circulating blood (148). It has been established that mature ECs can proliferate and migrate to repair the damaged cells, regardless of whether EC loss is minimal (a single or a few cells loss) or severe (after angioplasty) (8).

Accumulating evidence indicates that EPCs contribute to endothelial repair through direct engraftment and indirect release of angiogenic factors (101). However, the degrees by which EPCs are involved in the repair are different in various situations. For instance, in a hyperlipidemia-induced endothelial damage model, 3% to 5% of total cells on lesion-prone sites are Sca-1 and c-kit positive at 32 weeks in $ApoE^{-/-}$ mice, whereby bone marrow-derived EPCs contributed to 1% to 2% of total proliferating ECs at 10 months after bone marrow transplantation (35). Conversely, Hagensen *et al.* (44) did not find significant engraftment of bone marrow EPCs into the endothelial layer of isograft vessels in $apoE^{-/-}$ mice. With a Tie2/LacZ mouse chimeric model, it was shown that approximately 30% of ECs are derived from stem cells in allograft vessels (56), whereas most cells had recipient origins in vein isografts (150). In carotid injury mice with GFP-marked bone marrow transfer, about 9% of proliferating ECs were GFP and von Willibrand factor (vWF) double positive (128). In transplant vessels, published data indicate that bone marrow–derived stem cells contribute to the regeneration of the endothelium of allografts, but the percentage of stem cells reported to incorporate into the damaged vessel is variable (33, 50, 54, 56, 90, 102, 124). Collectively, these data suggest that EPCs can contribute to significant endothelial repair in severely damaged vessels as compared with "normal" vessel repair. The differences across the studies may be due to the variability in the cell markers used to identify the EPCs, as well as differences in animal models that were used (Table 1).

Logically, if EPCs can repair severely damaged endothelium, we should be able to see the effect of EPC therapy on vessel-function improvement. Abundant evidence indicates the positive result of progenitor cell therapy (3). For instance, in an elegant study by Wassmann and colleagues (136), a marked improvement was seen in endothelial function after systemic transfusion of vascular progenitor cells. The intravenous transfusion of spleen-derived mononuclear cells

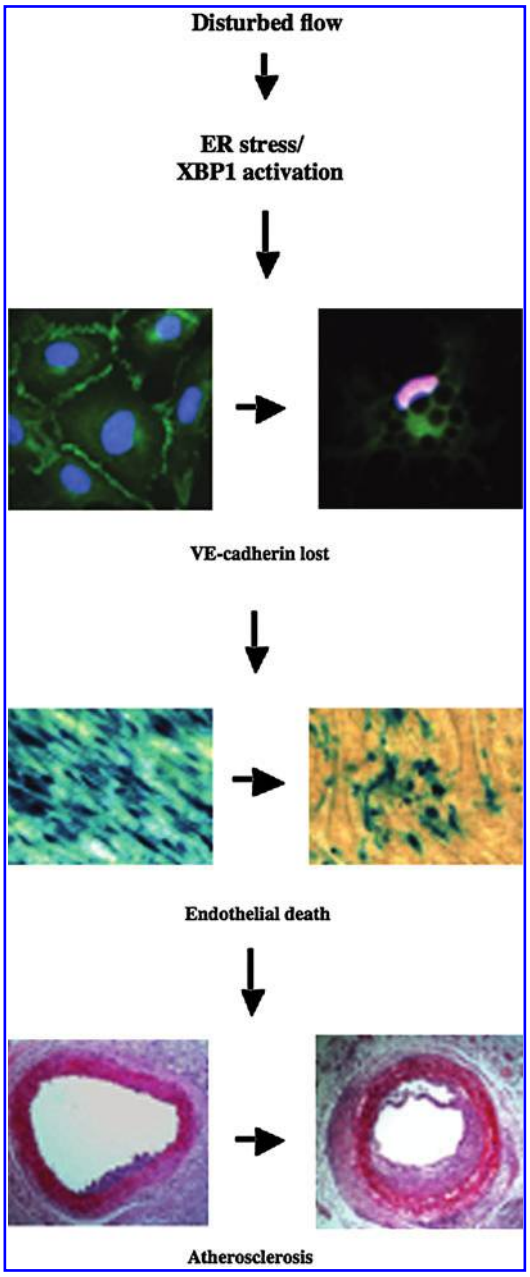

FIG. 3. Schematic figure represents a potential pathway from disturbed flow stimulation to the development of atherosclerosis, in which XBP1 activation in the endoplasmic reticulum (ER) is a key event. XBP1 can serve as a transcription repressor to downregulate VE-cadherin expression, resulting in caspase activation and endothelial apoptosis, which initiate the formation of atherosclerosis (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

isolated from wild-type mice significantly restored and improved endothelium-dependent vasodilatation in apoE$^{-/-}$ mice fed with a high-cholesterol diet. The intravenously transfused EPCs engraft specifically at sites of injury, subsequently enhancing re-endothelialization and reducing neointima formation (138). No doubt EPC-based technologies and strategies to enhance the function and number of ECs have gained considerable interest in the field of interventional cardiology. Contrary to these observations, a separate study showed that EPC-mediated re-endothelialization markedly increased neointimal lesions in a porcine model, in which stainless steel stents coated with anti-CD34 antibody were used to capture circulating EPCs (CD34$^+$ cells) (110). In a Phase I clinical trial, 16 patients with *de novo* coronary heart disease were successfully treated with implantation of the EPC-capture stents (5). Nevertheless, the possibility that EPCs may also contribute to unfavorable conditions, such as plaque progression and instability, suggests precautions and careful consideration about using these cells for vascular therapy. Some evidence shows that plaque rupture and hemorrhage are closely related to the density of microvessels within the vessel wall, especially in the adventitia (*i.e.*, vasa vasorum) (96). Increased numbers of early EPCs may promote atherosclerosis by augmenting disease-associated angiogenesis. Pula *et al.* demonstrated that early EPCs secrete angiogenic cytokines, such as thymidine phosphorylase, that play important roles in promoting endothelial migration and angiogenesis *in vivo* (101). In addition, the transfer of spleen cell–derived EPCs and bone marrow cells accelerated atherosclerosis in ApoE knockout mice, therefore indicating a proatherogenic effect of the cells (38). Taken together, these results indicate that, although circulating EPCs repair and rejuvenate the arteries under physiologic conditions, they can also contribute to lesion formation under specific pathologic conditions. EPCs may protect against lesion formation at early stages but decrease plaque stability in advanced lesions and promote atherosclerotic plaque progression in ischemic settings (76). Therefore, EPCs should be used carefully in therapeutic settings, especially in those patients with severe atherosclerosis.

SMC Accumulation in Neointima Induced by Progenitor Cells: Bad Guys?

SMC accumulation in the intima is a key event in the development of arteriosclerosis (107). As discussed earlier, two possible sources of SMCs in the lesion are mature SMCs of the vascular media and progenitor cells from the vessel wall and circulating blood. Although the precise frequency and roles of progenitor cell-derived SMCs in arteriosclerosis remain debatable, it is widely accepted that the progenitors can contribute to SMC accumulation in lesions, depending on the differential degrees of vessel damage (Fig. 4). Controversies concerning progenitor cell frequency are likely to arise because of the difficulty of tracking these cells *in vivo* and the variation in animal models used from one laboratory to another. In native atherosclerosis, SMCs in atherosclerotic plaques were shown to originate from bone marrow progenitors (eGFP$^+$ or β-galactosidase$^+$ labeled), thereby implying that the SMCs were derived from hematopoietic stem cells (115, 117). In humans, Caplice and colleagues (20) found that 20% of SMCs in coronary atherosclerotic plaques of deceased patients who had undergone a sex-mismatched bone marrow transplantation for hematologic disease had originated from the transplanted bone marrow cells (20). Whereas other studies show that hematopoietic stem cells could give rise to arterial SMCs after injection into the border zone of experimental myocardial infarcts in mice (45), some investigators failed to identify bone marrow–derived SMCs in atherosclerotic

Table 1. Endothelial Cell Origin in Grafted Vessels

Species	*BM transfer*	*Graft*	*Method*	*Recipient*	*Donor*	*Bone marrow*	*Reference*
Mouse	BM chimera	Vein/auto	TIE2/LacZ	~95%	0	~30%	Xu *et al*. (150)
Mouse	BM chimera	Carotid/iso	GFP	0	100%	0	Hagensen *et al*. (44)
Mouse	BM chimera	Aorta/allo	TIE2/LacZ	>95%	<5%	~30%	Hu *et al*. (56)
Mouse	BM chimera	Aorta/allo	GFP	Majority	NA	~20%	Feng *et al*. (33)
Rat	sex-mismatch	Heart/allo	Y-PCR	~100%	0	<5%	Hillebrands *et al*. (50)
Human	sex-mismatch	Heart/allo	Y-probe	NA	95%	NA	Hruban *et al*. (54)
Human	sex-mismatch	Heart/allo	Y-probe	42%	58%	NA	Quaini *et al*. (102)
Human	sex-mismatch	Heart/allo	Y-probe	24.3%	NA	NA	Minami *et al*. (90)
Human	sex-mismatch	Heart/allo	Y-probe	1–24%	NA	NA	Simper *et al*. (124)

BM, bone marrow; GFP, green fluorescent protein; NA, not available ; Y-PCR, Y-chromosome–specific polymerase chain reaction; Y-probe, Y-chromosome–specific probe.

lesions (13). Such diverse results may be due to the variation in experimental methods that were used (*e.g*., accuracy of double staining). Nevertheless, it is noteworthy that vessel wall–resident progenitor cells may also give rise to SMCs. Benditt and Benditt (11) established a monoclonal theory of SMCs in lesions whereby smooth muscles were observed to display monoclonal origin (*i.e*., derived from a single cell) (11). According to this theory, SMCs in arteriosclerotic lesions could originate from one stem/progenitor cell that is present in the arterial wall. Subsequent studies demonstrated that the arterial wall contains stem cells that can differentiate into SMCs [for review, see (84)].

In vein graft–induced atherosclerosis, the contribution of progenitor cells seems to differ from that of native atherosclerosis. The earliest cellular event in mouse vein grafts is cell death, as demonstrated by markedly increased apoptotic and necrotic ECs (87). Studies indicate extensive loss of ECs in the intima during early stages of human vein grafts (26). After the vascular cell death is progenitor cell regeneration, mononuclear cell infiltration (163), and SMC accumulation, all of which contribute to the formation of arteriosclerotic lesions (147). Although the vessel-wall cells contribute to lesion formation in graft atherosclerosis, recent evidence shows that SMCs in the neointima are derived from progenitor cells of both the vessel wall and circulating blood (57). In a recent report of isografted artery, authors did not find the engraftment of bone marrow–derived smooth muscle cells (12). Moreover, bone marrow progenitor cells were found to attach to the endoluminal surface of the injured segments, thereby resulting in the narrowing of the lumen and contributing to the formation of neointima (19). In contrast, no SMCs derived from progenitor cells were seen in uninjured femoral arteries of control mice. From these observations, it is tempting to postulate that the frequency of progenitor cell–derived SMCs that appear in the lesions depends on the severity of vessel injury (Fig. 4).

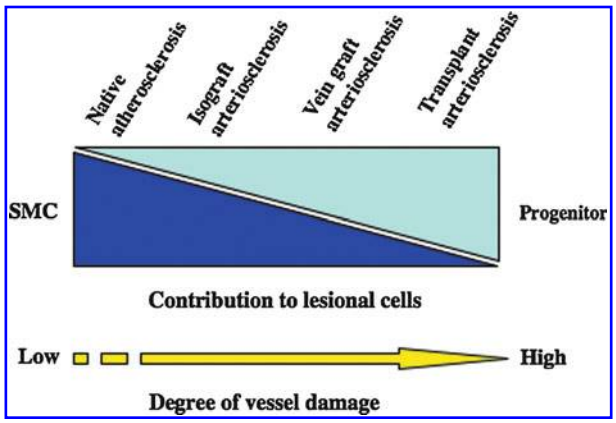

FIG. 4. The frequency of progenitor cells contributing to the endothelial repair and accumulation of smooth muscle cells in the lesions is largely dependent on the degree of vessel damage. The more severe the damage, the more progenitor cells are involved (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Transplant-accelerated arteriosclerosis in arteries is a major limitation to the long-term survival of patients with solid-organ transplantation. Transplant arteriosclerosis is characterized by a diffuse, uniform, and concentric narrowing of the artery as a result of the proliferative and fibrocellular intima (104). A hallmark of lesion formation is mononuclear cell infiltration into the vessel wall of grafts at an early stage, followed by neointimal formation that is largely constituted by SMCs (137). The vessel-transplantation model is an appropriate method to study the issue of SMC origin in the neointima. Recent data obtained from various studies indicated variable origins of SMCs in transplant arteriosclerosis (Table 2) (45, 50, 55, 57, 74, 112, 115, 121). Several animal experiments of aortic and cardiac transplantation demonstrated that neointimal SMCs of both aortic/cardiac allografts and lesions were of recipient and not donor origin (50, 115, 121). Similarly, SMCs in neointima in human cardiac allografts were found to be recipient derived, but at variable percentages (41, 102). In comparison to cardiac allografts, Grimm *et al.* showed that in renal allografts, 60% to 80% of neointimal SMCs were of recipient origin (42). Analysis of the reciprocal combinations, however, clearly demonstrated the persistence of a population of recipient-type cells in humans (41). Altogether, it is very likely that SMCs in transplant arteriosclerosis of mouse and rat models are of recipient progenitor cell origin, whereas SMCs in human transplants are derived from both donors and recipients (Table 2). Although the mouse and rat offer an incredibly valuable tool for the study of transplant arteriosclerosis in the laboratory, it is essential for the investigator to be aware of similarities and differences that exist between animal models and human disease (147).

Table 2. Smooth Muscle Cells in Neointima of Grafted Vessels

Species	*BM transfer*	*Graft*	*Method*	*Recipient*	*Donor*	*Bone marrow*	*Reference*
Mouse	BM chimera	Vein/auto	LacZ	40%	60%	None	Hu *et al*. (57)
Mouse	BM chimera	Carotid/iso	GFP	0	100%	0	Bentzon *et al*. (12)
Mouse	BM chimera	Aorta/allo	Y-probe	Majority	None	None	Li *et al*. (74)
Mouse	BM chimera	Aorta/allo	LacZ	>95%	NA	10.8%	Shimizu *et al*. (121)
Mouse	BM chimera	Heart/allo	LacZ/GFP	~88%	NA	~82%	Sata *et al*. (115)
Mouse	BM chimera	Aorta/allo	SM22/LacZ	95%	None	None	Hu *et al*. (55)
Rat	sex-mismatch	Heart//allo	Y-PCR/SMA	>95%	None	None	Hillebrands *et al*. (50)
Human	sex-mismatch	Heart/allo	Y-probe	<5%	NA	NA	Hruban *et al*. (54)
Human	sex-mismatch	Heart/allo	Y-probe	16%	NA	NA	Glaser *et al*. (41)
Human	sex-mismatch	Heart/allo	Y-probe	60%	NA	NA	Quaini *et al*. (102)
Human	sex-mismatch	Heart/allo	Y-probe	<5%	NA	NA	Minami *et al*. (90)
Human	sex-mismatch	Kidney/allo	Y-probe	60–80%	NA	NA	Grimm *et al*. (42)

BM, bone marrow; GFP, green fluorescent protein; NA, not available ; Y-PCR, Y-chromosome–specific polymerase chain reaction; Y-probe, Y-chromosome–specific probe.

If progenitor cells contribute to SMC accumulation in restenosis, they could be taken as "bad guys." Strikingly, several reports, however, demonstrated that smooth muscle progenitor therapy could provide a potential benefits instead. For instance, the injection of smooth muscle progenitor cells was shown to reduce the progression of early atherosclerotic plaques in advanced atherosclerotic mice, thus suggesting that the recruitment of these smooth muscle progenitor cells can promote plaque stabilization (162). Additionally, patients with acute coronary syndrome had reduced numbers of peripheral blood–derived progenitor cells that express smooth muscle markers as compared with patients with stable angina and healthy subjects (123). Interestingly, Simper *et al*. (122) demonstrated that smooth muscle progenitor cells derived from circulating blood expressed a distinct range of extracellular matrix and matricellular proteins that were unique to SPCs and aortic SMCs; the cells, however, produced less proteases and inflammatory cytokines (122), therefore suggesting that circulating smooth muscle progenitor cells may play a beneficial role in suppressing atherosclerosis or stabilizing the plaque.

Common Progenitors: A Double-edged Sword

As described earlier, progenitor cells can either protect/repair the endothelium and promote plaque stabilization, or participate in the pathogenesis of arteriosclerosis by SMC accumulation and inducing narrowing of the lumen. An even more complicated situation is that a common progenitor may exist that can differentiate into ECs, SMCs and macrophages. Whereas several groups have demonstrated the capacity of blood-derived $CD34^+$ progenitor cells to form foam cells, ECs, and SMCs (25, 126), others have found that embryonic stem cell–derived $Flk\text{-}1^+$ and $Sca\text{-}1^+$ progenitors can differentiate into both ECs and SMCs in response to different growth factors, such as VEGF and PDGF (154). Similarly, laminar flow has been shown to induce ESC differentiation toward EC lineages, while suppressing SMC differentiation (159). Interestingly, mesenchymal stem cells also possess the capacity to differentiate into both ECs and SMCs (135) and vascular progenitor cells are able to differentiate into contractile-type smooth muscles in the absence of VEGF or into synthetic-type phenotypes in the presence of PDGF-BB, respectively (93). Other hypotheses suggest the existence of common precursors of endothelial and mural cells, based on the isolation of several embryonic populations that showed the ability to differentiate into both endothelial and mural cell lineages when stimulated with VEGF or PDGF, respectively. Initially, VEGFR2-positive vascular precursors, derived from mouse embryonic stem cells, and more recently, from mouse and human iPS, showed plasticity toward endothelial and mural lineages (92–94). Alternatively, similar progenitors can be obtained performing isolation of $Sca\text{-}1^+$ cells from mouse stem cells; when exposed to differentiating stimuli, they give rise to EC and mural cells (143). In addition, human embryonic cell–derived $CD34^+$ cells were induced to differentiate into ECs as well as SMCs expressing SMC markers when cultured with PDGF-BB and TGF-β, indicating plasticity (49). Mature ECs have also been shown to be capable of transdifferentiation into SMCs. Furthermore, the immunosuppressive drug – cyclosporine has been shown to inhibit both endothelial and smooth muscle progenitor cell growth, thus attenuating neointimal formation in transplant models (27). Therefore, it is crucial for us to understand the molecular mechanisms of vascular progenitor cell differentiation, by which we can potentially guide the direction of the cell fate needed for the beneficial effect on patients with arteriosclerosis.

Endothelial Differentiation

An abundance of evidence shows that both adult and embryonic progenitors can differentiate into ECs *in vivo* and *in vitro*, although the signal pathways leading to cell differentiation are yet to be fully elucidated. Both VEGF and its receptor are believed to play critical roles in endothelial differentiation. VEGFR2 is the earliest marker of angioblast precursors, specifically marking a subset of Brachyury-positive cells that migrate into the extraembryonic yolk sac to form the vascular plexus during murine development. A deficiency in VEGF-A or a lack of either VEGF receptors (*i.e*., VEGFR1 and VEGFR2) resulted in failure of vascular development in embryos (22, 34, 119). In a study involving bone marrow–derived multipotent progenitor cells, it was proposed that the mechanism driving their differentiation toward ECs in the presence of VEGF165 was dependent on activation/phosphorylation of the MAPK/ERK signaling (146) (Fig. 5).

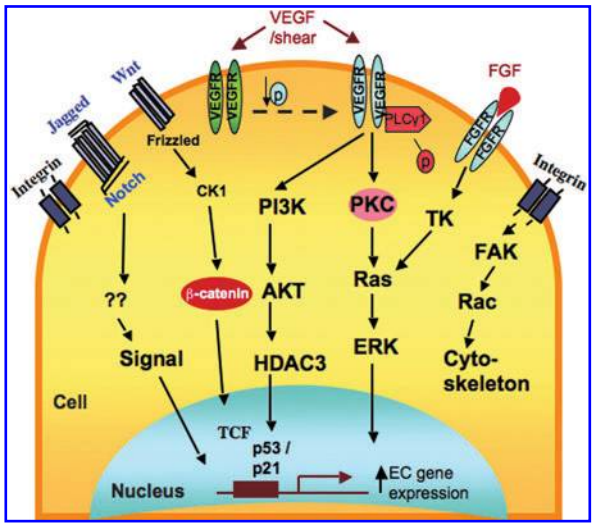

FIG. 5. VEGF and other factors influence progenitor differentiation into ECs. VEGFR2-PLCγ1 signaling relays a major phosphorylation site in VEGFR2 and the downstream Ras signaling pathways to promote the expression of endothelial markers in differentiating stem cells. The presence of FGF stimulates a signaling shared with VEGF pathways. In addition, activated Notch signaling and enhanced Wnt-induced EC differentiation from progenitor cells are also crucial. Shear stress upregulates HDAC3 via Flk-1-PI3K-Akt signal pathways and mediated p53 deacetylation and p21 activation, which are crucial for shear- and VEGF-induced EC differentiation (To see this illustration in color the reader is referred to the web version of this article at www .liebertonline.com/ars).

The phosphorylation of the intracellular domain of VEGFR2 and its interactions with phospholipase Cγ1 and PI3K are fundamental for the survival/proliferation of hESC-derived Flk1 progenitors and their commitment to the endothelial lineage (113). The VEGF effect on EC differentiation is not simply an on/off mechanism, but involves fine regulation. Embryos overexpressing VEGF-A by two- to threefold show abnormal heart formation and die at E12.5 to E14 (91). The high-affinity VEGFR1 regulates VEGF signal intensity on VEGFR2 by sequestering the growth factor from the environment. Although Flt1-deficient mice die at midgestation because of vascular disorganization, tyrosine kinase–deficient mice in which VEGF can bind to VEGFR1 but cannot produce downstream signaling were found to develop normally (51). Neuropilin 1 is expressed on the surface of ECs and, together with VEGFR2, forms a specific receptor for VEGF-165. It has been demonstrated that protein kinase A activation leads to overexpression of VEGFR2 and neuropilin 1, thereby increasing the sensitivity of VEGFR2$^+$ progenitors to VEGF-165 and inducing differentiation (152). Interestingly PKA is upregulated by shear stress (24), a condition that has been shown to stimulate EC differentiation of VEGFR2-positive cells by upregulating VEGF receptors (153).

Endothelial cell differentiation also is dependent on the fibroblast growth factor (FGF) family group of proteins, with the most studied being basic FGF (b-FGF or FGF-2). Knockdown of either of the genes coding for FGF receptors 1 and 2 leads to lethality before gastrulation (28, 151), whereas bFGF-depleted embryos developed normally but showed decreased vascular tone and low blood pressure, thus indicating a certain degree of redundancy in the FGF family (31). The mechanism of FGF2 stimulation of EC differentiation was dependent on Src kinase activity, whereas the inhibition of MEK and MAP kinase and PKCγ, two pathways previously implicated in EC differentiation, did not prevent the FGF2-stimulating effect (67).

Integrin receptors play a major role in extracellular matrix cell signaling by binding to the matrix proteins for the execution of bidirectional signaling. Integrins can regulate the stabilization, migration, and structural integrity of most types of cells (100). Integrins are also involved in the development of vessels, as $\alpha_v\beta_3$ integrins were found to be upregulated in angiogenesis, whereas a deletion of the α_5 chain reduced capillary plexus formation (36). At the same time, β_1 integrins have been shown to be necessary for teratoma growth and vasculogenesis in embryoid bodies (14), and an endothelium-specific deletion of β_1 integrins results in vascular defects and early embryonic lethality of mice (73). Consistent with these observations, Malan *et al*. (82) showed that the sprouting and vascular-network formation of endothelial cells from β_1 integrin$^{-/-}$ embryoid bodies were defective and markedly reduced, likely because of impaired EC differentiation. Another extracellular matrix protein, laminin, also has been demonstrated to be involved in EC differentiation of murine embryonic bodies through the synergistic induction of FGF2 transcription (30).

Wnt proteins have many important roles during development, including a fundamental role in the formation (*in vivo* and *in vitro*) of the primitive streak, where hemangioblasts are generated (37, 78). In particular, the addition of a Wnt-pathway inhibitor, Dickkopf1, was found to cause a marked reduction in the generation of CD34$^+$ and Flk1$^+$ cells in human stem cell–derived embryoid bodies, whereas the supplementation of Wnt1 enhanced EC differentiation (139). Similarly, Wnt5a$^{-/-}$ stem cells and embryos were not able to commit to endothelial lineage; adenovirus-mediated supplementation of Wnt5a reversed the phenomenon, acting through the activation of β-catenin and PKCα signaling (155). Conversely, Notch signaling plays fundamental roles in determining the fate of a variety of cell types. Four Notch receptors (Notch1–4) and five Notch ligands (Jagged1, 2, and Delta-like1, 3, and 4) have thus far been identified in mice and humans (120). The activation of the Notch receptor by its ligands leads to its cleavage by γ-secretases, nuclear translocation of its intracellular domain, and subsequent activation of various downstream genes, such as those of the HES and HEY families (61). Notch plays an important role in embryonic vascular development, particularly in arteriovenous differentiation (70). A loss of Notch has been shown to result in the failure of developing zebrafish to express arterial markers (71). Furthermore, the constitutive activation of Notch has been shown to suppress the expression of a venous cell marker, Flt4. In stem cell–derived VEGFR2$^+$ cells, shear stress has been demonstrated to stimulate EC differentiation through ligand-independent VEGFR activation that leads to Notch cleavage through VEGFR-mediated signaling pathways (86).

Histone deacetylases (HDACs) are a class of enzymes composed of at least 17 genes; whereas ESCs express virtually all of the HDAC family genes, human peripheral blood–derived endothelial progenitors highly express HDAC1,

HDAC3, and SIRT1 (Xu, unpublished data). Zeng *et al*. (159) previously found that shear stress can rapidly activate the VEGF receptor-Akt-eNOS pathway in ESC-derived progenitors, in which Akt can also induce HDAC3 phosphorylation (159). Additionally, shear stress has been found to upregulate p53, the expression of which is also a downstream target for HDAC3 (159). Taken together, shear stress is a positive signal for stem/progenitor cell differentiation into ECs via pathways similar to those used by VEGF (Fig. 5).

SMC Differentiation

Accumulating evidence shows that smooth muscle progenitors derived from both adult and embryonic stem cells can differentiate into mature SMCs, through similar signal pathways. In this section, we discuss this issue both in embryonic development and in adult differentiation. The initial signal sensed by progenitor cells is through their cell-surface receptors: PDGF receptors, TGF-β receptors, and integrins (Fig. 6). Integrins consist of a family of heterodimeric transmembrane proteins that interact with different components of extracellular matrix proteins to transmit bidirectional signals between cells and the matrix proteins. To date, 18 α- and eight β-integrin subunits have been described, some of which can combine to form 24 different heterodimers (16). The activation of integrin receptors by tyrosine phosphorylation of β-subunits is essential for their function, whereby signal transmission through these complexes can affect various aspects of cell physiology, including SMC differentiation (16). The majority of studies on SMC differentiation highlight the dependence of this process on the interactions of α_1, β_1, and α_v integrins with collagen IV (142). After tethering to the collagen IV, progenitor cells require additional signal(s), such as TGF-β and PDGF-BB for the initiation of differentiation (141). Recent data showed that PDGF-BB and TGF-β are crucial for the differentiation into SMCs of vascular progenitor cells derived from the adventitia and media of the arterial wall (111, 142).

The downstream signal-transduction pathways leading to SMC differentiation involve focal adhesion kinases, PI_3-kinases, and mitogen-activated protein kinases. In addition, signals such as Nox4 activation (consequent of free radical generation) have also been shown to be crucial for SMC differentiation (140). NADPH oxidase (Nox) 4 activation is highly regulated by the cap-'n-collar family member NF-E2–related factor (Nrf) 3, the overexpression of which enhances SMC differentiation, whereas a knockdown inhibits their differentiation (98). Nrf3 mediates SMC differentiation by increasing the recruitment of both SRF and myocardin and their subsequent binding to the promoter of SMC genes. Moreover, Nrf3 is also involved in the regulation of Nox4 activation and various antioxidant gene expressions, which are important regulators of SMC differentiation (98).

More recently, Magariti *et al*. (83) showed that HDAC7 can mediate smooth muscle differentiation, which has also been shown to be important for endothelial migration and angiogenesis (95). Additionally, the growth factor TGF-β has the capacity modify α-actin gene expression through a control element that is present in the 5′-region of α-SMA, calponin, and SM22α (2, 47). In particular, TGF-β acts by decreasing the expression of Kruppel-like transcription factor KLF4, which binds to the same 5′-region, thereby negatively regulating the TGF-β–dependent SMC differentiation marker gene promoter activity (2, 77). In addition, TGF-β enhances SRF expression/activity and smooth muscle marker expression through a CArG element (47, 52). Alternatively, TGF-β induction of SMC differentiation has also been described to be dependent on the induction of Notch signaling through Jag-1. In addition, the activation of the Notch signaling pathway appeared to be sufficient for inducing SMC differentiation (69). Therefore,

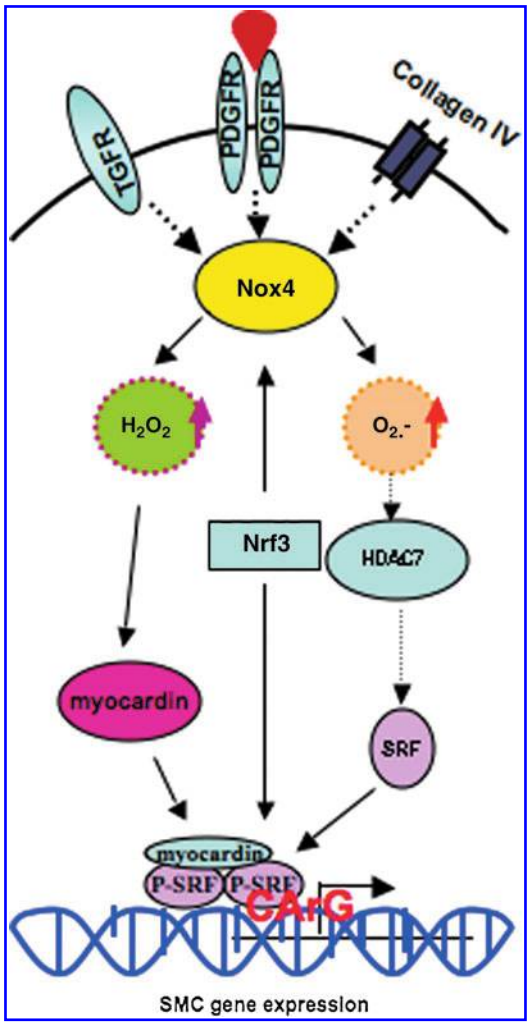

FIG. 6. Regulatory role of PDGF, TGF, and integrins in SMC differentiation. PDGF-BB and TGF-β1 can bind to their receptors in the presence of interactions between collagen IV and integrins, which indirectly induce Nox4 expression. Activated Nox4 generates ROS (H_2O_2 and O_2.). Nox4-derived H_2O_2 upregulates SRF gene transcription and protein translation, phosphorylates SRF in the cytoplasm, and drives activated SRF to translocate into the nucleus from cytoplasm. Phosphorylated SRF binds to CArG elements within the promoter-enhancer region of SMC-specific genes, recruits coactivator myocardin and other transcription factors, and then regulates SMC differentiation. Meanwhile, Nox4-derived O_2.$^-$ activates indirectly HDAC7, increases SRF-mediated gene transcription activation, and further drives SMC differentiation. Furthermore, Nrf3 is involved in both Nox4 expression and direct interaction with transcription factors for SMC gene expression (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

the activation of a signaling network within the cell is required in response to cytokine stimuli, thereby leading to the commitment of progenitor cells to the smooth muscle lineage (Fig. 6).

Summary and Perspectives

The dogma that describe circulating progenitor cells as a unique reservoir of stem cells that migrate throughout the body to regenerate and repair, both during normal homeostasis and under pathologic conditions, has recently been revisited because of the large body of work illustrating the importance of resident progenitor cells. Progenitor cells that reside in the vascular tissue, in particular, are likely to play a direct or indirect role(s) in the pathology of atherosclerosis. Intriguingly, recent evidence provides implications of an important link between smooth muscle, endothelial, and hematopoietic cells through their origins from common progenitors in embryonic and adult tissues (15). Furthermore, these vascular progenitor cells have the potential to differentiate either into ECs to repair damaged endothelium, or into SMCs to participate in neointimal lesions. Smooth muscle progenitors have a more heterogeneous and indefinite embryonic origin, which provides different sources for distinct SMC populations in the vessel wall (81). It is therefore plausible that the microenvironment in which the progenitor cells reside is an important determinant of their subsequent differentiation into either endothelial or smooth muscle cells. The signal pathways mediating EC differentiation mainly involve VEGF-PI3K-eNOS-HDAC3-p21, whereas SMC differentiation is largely mediated by integrin/collagen IV, PDGF, and TGF-β, all of which subsequently activate downstream signaling and transcription factor SRF, myocardin, and Nrf3. Although the elegant revelation of these signaling pathways of progenitor commitment into SMCs or ECs, undoubtedly many unanswered questions must be addressed to identify effective therapeutic interventions for atherosclerosis.

The different sources of progenitor cells contributing to the pathogenesis of arteriosclerosis have been repeatedly reported, but the frequency and degree of contribution of these cells remain controversial. For instance, how many cells are derived from the vessel-wall progenitors, and whether bone marrow progenitors are putative progenitors participating in vascular repair and SMC accumulation. Furthermore, the interaction between mature ECs or SMCs in the vessel wall and progenitors from a variety of sources could be crucial in determining the lesion development; the availability of such data is, however, lacking. The second important issue is whether a common progenitor exists in the vessel wall or in circulating blood, which may differentiate into both ECs and SMCs, depending on the local microenvironment, as mentioned earlier. Finally, what molecular mechanisms determine the direction of progenitor cell differentiation? If we could answer these questions, we could manipulate vascular progenitor cells to control their trafficking to areas of damaged endothelium to prevent the formation of atherosclerosis. Thus, in the future, investigators may arbitrarily "educate" the progenitors in becoming the good guys for the vessel wall.

Acknowledgments

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Address correspondence to:
Prof. Qingbo Xu
Cardiovascular Division
King's College London BHF Centre
125 Coldharbour Lane
London SE5 9NU
United Kingdom

E-mail: qingbo.xu@kcl.ac.uk

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Abbreviations Used

ABCG = ATP-binding cassette subfamily G member
EC = endothelial cell
eNOS = endothelial nitric oxide synthase
EPC = endothelial progenitor cell
FGF = fibroblast growth factor
GFP = green fluorescent protein
HDAC = histone deacetylase
KLF = Kruppel-like transcription factor
MAPK = mitogen-activated protein kinase
Nox = NADPH oxidase
Nrf = the cap-'n-collar family member NF-E2–related factor
PDGF = platelet-derived growth factor
PKC = protein kinase C
SMA = smooth muscle actin
SMC = smooth muscle cell
SPC = smooth muscle progenitor cell
SRF = serum response factor
TGF = transform growth factor
VEGFR = vascular endothelial growth factor receptor

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