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The Role of Progenitor Cells in the Development of Intimal Hyperplasia

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Neointimal hyperplasia in response to arterial injury is a complex process, classically believed to be the consequence of vascular smooth muscle cell proliferation and migration, and the synthesis of extracellular matrix [1, 2]. Recently, it has been proposed that the neointimal lesion also consists of progenitor cells attracted to the site of vascular injury. In this review, we will summarize the reports that demonstrate an important role for progenitor cells in the development of intimal hyperplasia. We will also examine the involved cell types as well as the mechanisms underlying progenitor cell recruitment to the injured arterial wall.

Bone marrow derived progenitor cells contribute to the neointimal lesion after arterial injury

The contribution of bone marrow derived cells to the neointimal lesion has been demonstrated repeatedly using bone marrow transplant in conjunction with mouse models of vascular injury [3–6]. One of the earliest studies of this phenomenon was performed by Sata *et al* using bone marrow cells, which express β-galactosidase (a product of the LacZ gene), from ROSA26 mice. Cells from these mice are easily identified as blue when stained with X-gal [3]. The techniques were as follows. Wild type mice were lethally irradiated, then injected via tail vein with bone marrow cells derived from ROSA26 mice. After confirmation that the ROSA26 bone marrow cells had reconstituted the bone marrow and blood cell lines of the wild type mice, a wire injury of the femoral artery was performed. Histological analysis of the subsequent neointimal lesion at specified time points revealed the presence of LacZ positive, or transplanted bone marrow derived cells. Furthermore, double staining revealed that the LacZ positive cells had differentiated into both smooth muscle (α-smooth muscle actin or α-SMA positive) as well as endothelial ($CD31^+$) like cells. As shown in Table 1, numerous subsequent studies have confirmed the existence of bone marrow derived cells in the neointima. While in most of these studies the mouse bone marrow transplant model was employed, differing methods of labeling bone marrow cells as well as different types of injury have contributed to a wide range of values for the percentage of neointimal cells that are of bone marrow origin (ranging from 20 to 66%)[4, 5]. Furthermore, circulating white blood cells in an animal after bone marrow transplant are also bone marrow derived. As has been previously demonstrated, these inflammatory cells, specifically macrophages, can contribute to the neointimal lesion. Thus, it is important to evaluate the neointima in these models with specific staining to determine whether bone

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marrow derived cells have progenitor cell, smooth muscle cell, endothelial cell, or white blood cell markers.

The progenitor cell contribution to the neointima appears to be determined by the type of injury

Whereas the mouse wire injury model was used in all of the studies cited in Table I, it has been shown that the contribution of bone marrow derived progenitor cells to the arterial wall may vary depending on the type of injury [4]. Using a model that transplanted bone marrow cells from a transgenic mouse that expresses GFP (GFP^+ mouse) to a wild type mouse, Tanaka *et al* showed that three distinct types of mechanical injury produced varying degrees of bone marrow derived cell contribution to the arterial wall. In the first injury model, a 0.38mm straight spring wire was inserted into the mouse femoral artery to denude and dilate the artery. This model best recapitulates angioplasty procedures in humans, since it involves both vessel wall dilatation and endothelial denudation. In the second model, a polyethylene tube was placed around the mouse femoral artery (perivascular cuff induced injury). In the last model, the mouse common carotid artery was ligated just proximal to the bifurcation. Wire injury led to large numbers of GFP^+ cells in both the media and the neointima, whereas perivascular cuff placement and carotid artery ligation resulted in significantly fewer GFP^+ medial and neointimal cells. (Table 2) The authors also studied the fate of the bone marrow derived cells by examining α-SMA expression. Whereas a significant number of GFP^+ cells in the neointima and the media after wire injury were also α-SMA^+, only a few of the GFP^+ cells expressed α-SMA in the other two injury models [4]. Finally, the authors showed that while each mode of injury induced differing degrees of inflammation, the degree of inflammation did not correlate with the contribution of bone marrow derived cells to the neointima. In all models, inflammatory cells were predominately macrophages, with the greatest infiltration of macrophages reported after perivascular cuff placement. At 4 weeks after injury, few macrophages were detected in the lesions produced by wire injury or carotid ligation, even though these modes of injury resulted in the greatest number of bone marrow derived cells in the arterial lesion.

The findings of these studies clearly demonstrate that bone marrow cell contribution to arterial lesions can vary widely depending on the animal model of arterial injury. Similarly, previous studies of human vascular lesions suggest that the cellular constituents of vascular lesions also vary with the type of injury, for example, atherosclerosis vs. restenosis or restenosis after balloon angioplasty vs. vein graft intimal hyperplasia after vascular bypass [7, 8]. In humans, the specific contribution of progenitor cells to vascular disease is less well defined. Stem cell therapy for vascular disease in humans is currently being tested in clinical trials, [9, 10] even though it has never been proven with the elegance of the animal models that progenitor cells actually contribute to the arterial wall response to injury. The animal models, however, remain crucial in achieving a better understanding of the mechanisms underlying progenitor cell recruitment. In this review, we focus specifically upon mouse models of vascular injury that result in intimal hyperplasia and restenosis. However, it is important to note that none of these models precisely recapitulate the human disease process.

Characterization of the progenitor cells that contribute to animal models of intimal hyperplasia

Bone marrow cells

In the foregoing mouse models of restenosis, the entire contents of the bone marrow were transplanted into the recipient mouse. Bone marrow is primarily composed of cells of the

blood cell lineages (e.g. myelocytes, lymphocytes, monocytes, megakaryocytes, erythrocytes) at various stages of differentiation. *Stem cells*, defined as cells that are capable of self-renewal and differentiation, constitute in the human only approximately 0.01% of the total bone marrow. Together, *stem cells* and also *progenitor cells* (cells that are more differentiated) account for approximately 0.1% of total bone marrow cells. Thus, it is only a small percentage of bone marrow cells that are capable of directly repopulating the neointimal or endothelial layers after arterial injury.

In humans, stem cells have been classified broadly into *hematopoietic stem cells* (HSC) (CD34+/CD38−) or non-hematopoietic or *mesenchymal stem cells* (MSC) (Figure 1) [11]. HSCs, which differentiate into all the blood cell lines, are believed to be derived from a very early embryonic precursor – the hemangioblast. Of note, many believe that the hemangioblast is a common precursor for both HSCs and *endothelial progenitor cells* (EPC), although this point remains controversial.[12–14] EPCs, however, are thought to be important in adult vasculogenesis and, relevant to this review, may also participate in the neointimal lesion. MSCs, on the other hand, have been described to differentiate into muscle cells, osteoblasts, chondrocytes, or adipocytes. Important cell surface markers that distinguish between the different types of progenitor cells are summarized in Table 3. In the following sections we will review how both HSCs and MSCs may contribute to neointimal hyperplasia.

Hematopoietic stem cells

HSCs are defined by the properties of self-renewal and the ability to differentiate into cells of all blood lineages. Although the accepted marker for human HSCs is $CD34^+$, the gold standard for identifying these cells remains a reconstitution assay. Though impractical in humans, in the mouse a single HSC is capable of reconstituting the entire hematopoietic system for the life of the animal[15, 16]. In the mouse, HSCs are characterized by the combination of various markers, including c-kit^+/sca-1^+/lineage depleted, and are often referred to as the KSL cell population.

Some authors have proposed that HSCs can differentiate into muscle cells (including smooth and skeletal muscle as well as myocardial cells), neural cells, hepatocytes, as well as epithelial, kidney, intestinal and pancreatic cells [17–19]. Moreover, early studies by Sata *et al.* have demonstrated that HSC's are important in the bone marrow's contribution to the neointima [3]. HSCs, or KSL cells, were isolated from ROSA26 mice and just these cells, rather than the entire bone marrow, were transplanted into wild type mice. Mice then underwent femoral artery wire injury and the resulting neointimal lesion was shown to contain $LacZ^+$ cells, some of which were also α-SMA positive. The authors therefore concluded that the HSC fraction of bone marrow could give rise to not only hematopoietic cells, but also vascular cells involved in intimal hyperplasia. Despite these findings, the ability of HSCs to differentiate into non-hematopoietic cells (i.e. SMCs) remains controversial [20]. In studies where the mouse bone marrow has been reconstituted with a *single* HSC, transdifferentiation of HSCs into non-hematopoietic cells is extremely rare [21–23]. In fact, when Sata's group repeated their initial experiments using a single HSC to reconstitute the bone marrow rather than (c-kit^+/sca-1^+/lin^-) cells, they found very few cells in the neointima that were the progeny of the single transplanted HSC, suggesting that the population of c-kit^+/sca-1^+/lin^- cells used in their original experiments may have been contaminated with other progenitor cells [3, 20, 23].

Endothelial progenitor cells

EPCs were first isolated by Asahara *et al*. from human peripheral blood $CD34^+$ cells [24]. Asahara found that after 7 days of culture on fibronectin coated plates, not only were the

cells morphologically different from freshly isolated $CD34^+$ cells, but there was also a significantly higher percentage of cells that exhibited endothelial cell markers including CD31, Flk-1, Tie-2, or E-selectin. EPCs are currently defined as cells that express both progenitor and endothelial cell markers. In human cells, these markers would be $CD34^+$ (progenitor) and $VEGFR2^+$ (for endothelial cells) [25].

In the biology of intimal hyperplasia, it is believed that a faster rate of re-endothelialization after an arterial injury that results in intimal denudation, can reduce the formation of intimal hyperplasia [26]. Werner *et al*. reported that bone marrow derived progenitor cells contributed to the endothelial layer after mouse carotid wire injury [27]. Using retroviral infection to label bone marrow cells with a virus expressing GFP, these authors showed that up to 10% of ECs (vWF^+ cells) were also GFP+, and therefore of bone marrow origin. Follow-up studies by this group reported that mouse spleen-derived mononuclear cells could differentiate into cells with characteristics of EPCs. Intravenous injection of these mouse-derived EPCs after wire carotid injury accelerated re-endothelialization and decreased neointimal hyperplasia, thus suggesting that EPCs may indeed play an important role in regulating neointimal formation [28].

Mesenchymal stem cells

In contrast to hematopoietic stem cells, there is also a population of non-hematopoietic stem cells or mesenchymal stem cells (MSC) that are believed to originate from bone marrow stromal cells and differentiate into myocytes, osteoblasts, chondrocytes, and adipocytes [11]. Human MSCs typically express several cell surface markers such as CD105, CD44, CD90, CD71, and Stro-1, although none of these are specific to MSCs [11]. MSCs are, however, distinct from HSCs and other hematopoietic cells in that they generally do not express CD34 and CD45. Since there are no reliable MSC markers, these cells are often isolated using specific cell culture conditions. Typically, a single-step purification method using adherence to plastic cell culture plates is employed. This results in a population of fibroblast-like cells, which are characterized as MSCs based on their ability to differentiate into multiple mesenchymal lineages (e.g. osteogenic, chondrogenic, myogenic, etc.) [29]

Studies have shown that MSCs home to areas of injury after both site-directed and systemic administration [11]. MSCs have been studied extensively in the context of cardiac tissue repair and are currently one the of the cell types being studied in clinical trials of cardiac regeneration following myocardial infarction [30, 31]. The contribution of MSCs specifically to restenosis has only recently been explored [32]. Irradiated wild type mice underwent bone marrow transplantation with MSCs derived from GFP^+ mice. The mice were also transplanted via tail vein injection with whole bone marrow from GFP^- mice since MSCs alone would not be expected to fully rescue these mice from myeloablative doses of radiation. Two months after transplant and successful engraftment, femoral artery wire injury was performed. After four weeks, the injured vessels developed a significant amount of intimal hyperplasia containing GFP^+ cells (39±17%), indicating a robust contribution of bone marrow derived MSCs to this process.

Circulating progenitor cells

In addition to progenitor cells residing in the bone marrow, a constant small population of peripheral circulating progenitor cells has also been described [24, 33]. It is thought that this population of circulating progenitor cells can be isolated from peripheral mononuclear cells and have the ability to differentiate into other cell types, such as endothelial cells or smooth muscle cells[34, 35]. Simper *et al*. for example, demonstrated that smooth muscle cells could be derived from the peripheral blood of normal, healthy human subjects [34]. Specifically, these authors isolated mononuclear cells and cultured these cells in media containing PDGF-

BB which resulted in the induction of smooth muscle cell differentiation. Immunocytochemistry and western blot analysis of these cells revealed that they not only possessed typical SMC markers (α-SMA, smooth muscle myosin heavy chain, and calponin), but they also stained positive for the progenitor marker CD34 and the VEGF receptors (Flt1 and Flk1).

Similarly, Zhao *et al.* have shown that peripheral blood mononuclear cells can give rise not only to smooth muscle progenitor cells, but also pleuripotent stem cells [35]. They identified a subset of peripheral blood monocytes that display a fibroblast-like morphology but exhibit both monocyte (CD14) and HSC (CD34 and CD45) markers. These cells could be induced to differentiate into macrophages, T lymphocytes, epithelial cells, endothelial cells, neuronal cells, and hepatocytes [35]. Their findings suggest that progenitor cells that contribute to tissue repair after injury can be derived from the population of circulating cells and not necessarily directly from the bone marrow itself.

To further address whether circulating progenitor cells, as opposed to bone marrow derived progenitor cells, contribute to the arterial response after injury, Tanaka *et al*. developed a parabiotic model in which a GFP transgenic mouse was conjoined subcutaneously (no direct vascular anastamoses) with a wild type mouse [36]. These authors found as early as 10 days and up to 20 weeks after surgery, that 35–40% of circulating leukocytes in a wild type mouse were GFP^+. After femoral artery wire injury of the wild-type mouse, GFP^+ cells were detected in both the neointima (14.8±4.5%) and media (31.1±8.8%), thereby suggesting that a portion of the cells in the injured arterial wall were derived from the pool of circulating peripheral cells. Furthermore, GFP^+ cells found in the injured arterial wall were also shown to stain positively for CD31 and α-SMA, implying the presence of a cohort of circulating progenitor cells that has the potential to give rise to both endothelial and smooth muscle cells.

Mechanisms underlying progenitor cell recruitment to sites of arterial injury

Bone marrow derived progenitor cell recruitment to neointimal hyperplasia after vascular injury can be conceptualized in three stages: 1) mobilization of cells from the bone marrow, 2) migration and recruitment of bone marrow cells to the site of injury, and 3) differentiation of bone marrow cells into mature vascular cells, such as endothelial or smooth muscle cells. Cytokines and chemokines that have been shown to be important in these steps include but are not limited to Granulocyte Colony Stimulating Factor (G-CSF), Stromal derived factor-1α (SDF-1α), c-kit and c-kit ligand (KitL, also known as Stem Cell Factor or SCF), Matrix Metalloproteinase -9 (MMP-9), and Vascular endothelial growth factor (VEGF) as well as its receptor (VEGFR). In the following sections we will review each stage of progenitor cell recruitment and the current understanding of the important involved chemokines and cytokines.

Mobilization of progenitor cells from the bone marrow

It is believed that bone marrow progenitor cells (HSC or MSCs) are mobilized into the peripheral circulation in response to stress signals produced at the time of injury [37]. In the bone marrow, progenitor cells exist in a complex environment consisting of bone marrow stromal cells and extracellular matrix (ECM) rich in fibronectin, collagens, and various proteoglycans [37]. In order to exit the bone marrow, progenitor cells must migrate through a vascular barrier (bone marrow venous sinuses) that separates the hematopoietic compartment from the circulation. Quiescent progenitor cells are believed to be attached to bone marrow stromal cells or ECM through specific binding interactions, including VLA-4/

VCAM-1, SDF-1α/CXCR4, CD44/HA (hyaluronic acid), and interactions between P-E- and L- selectin [37]. For example, VCAM-1 is constitutively expressed by bone marrow endothelial and stromal cells, and disruption of the VCAM-1/VLA-4 interaction by antibodies to VCAM-1 or VLA-4 ultimately leads to progenitor cell mobilization.. [38, 39] Additionally, α4 and β2 integrins have also been shown to play a role in the interactions between progenitor cells and the bone marrow microenvironment, and defects in integrin expression have also lead to increased progenitor cell mobilization. [40, 41]

G-CSF has been well established as a mobilizer of stems cells in both humans and mice [42, 43]. Treatment with G-CSF leads to the accumulation of proteases, particularly neutrophil elastase and cathespin G, in the bone marrow and concurrent downregulation of their inhibitors. Neutrophil elastase and cathespin G, in turn, lead to cleavage of key adhesion molecules, including VCAM-1. Mobilization of progenitor cells by G-CSF has also been shown to be dependent on MMP-9, as Heissig *et al* have reported that G-CSF-induced progenitor cell mobilization was impaired in MMP-$9^{-/-}$ mice [44].

The possibility that treatment with G-CSF may increase intimal hyperplasia was suggested by the results of the MAGIC Cell trial, a randomized control clinical trial examining the effect of G-CSF mobilized peripheral blood stem cells in cardiac function after myocardial infarction and coronary stenting [45, 46]. The study found a trend towards increased restenosis in the patient cohort treated with G-CSF. This finding prompted follow-up studies of how G-CSF might affect neointimal hyperplasia in animal models. In a model of rabbit iliac artery stenting, Cho *et al* reported that at 60 days after stenting, rabbits treated with G-CSF developed significantly more intimal hyperplasia when compared to rabbits treated with placebo (0.34±0.04 vs. 0.26±0.04, p=0.015) [47]. Furthermore, the authors showed that at early time points after injury, treatment with G-CSF not only increased total peripheral white blood cell count, but specifically increased the number of putative EPCs ($CD31^+$, VE-$cadherin^+$, $CD34^+$, KDR^+) and smooth muscle progenitor cells (VE-$cadherin^+$/a-SMA^+ or $CD31^+$/a-SMA^+). Culture of these cells over 3 weeks with VEGF or PDGF resulted in endothelial (cobble stone shape and $CD31^+$) and smooth muscle like cells (hill and valley morphology and a-SMA^+), respectively. Therefore, increasing the number of mobilized progenitor cells may be beneficial to some types of injury (myocardial infarction), but may exacerbate others (arterial injury).

In addition to the effects of G-CSF, the interaction between SDF-1α and its receptor CXCR4 has also been shown to be important in regulating progenitor cell survival, cell cycle and mobilization [37]. Intravenous administration of exogenous SDF-1α as well as treatment with a specific CXCR4 inhibitor (AMD-3100) rapidly induces progenitor cell mobilization in both humans and mice [48]. Heissig *et al*. have shown that SDF-1α induces MMP-9 in the bone marrow, leading to cleavage of membrane bound KitL (mKitL) to soluble KitL (sKitL). This in turn results in increased progenitor cell cycling and enhanced cell motility, and ultimately leads to progenitor cell mobilization [5, 44, 48, 49]. MMP-9-induced cleavage of mKitL to sKitL has also been demonstrated to be essential in the formation of intimal hyperplasia. While vascular injury in MMP-$9^{-/-}$ mice resulted in minimal intimal hyperplasia, treatment with exogenous sKitL was found to “rescue” these mice and increase intimal hyperplasia by 2.5 fold [5]. Furthermore, mobilization of bone marrow derived cells, and consequently intimal hyperplasia, can be inhibited by administration of the drug Gleevec, a c-kit inhibitor [5].

Progenitor cell mobilization has been studied extensively in the context of wound healing and vasculogenesis. The data from these studies have shown that certain disease states may alter bone marrow cell mobilization in response to injury. Of note, these disease states are often the co-morbidities present in patients with peripheral vascular disease. Patients with

diabetes have recently been found to have fewer circulating progenitor cells, as demonstrated by peripheral blood analyses. [50–52] Both diabetes and advanced age have been shown to impair progenitor cell mobilization in mice. [53, 54] In their studies of wound healing in diabetic mice, Gallagher *et al*. have shown that impaired nitric oxide synthase (NOS) activation results in decreased EPC mobilization and therefore impaired vasculogenesis and wound healing. Hyperbaric oxygen therapy, by activating NOS, increases nitric oxide (NO) production and therefore increases EPC mobilization from the bone marrow. [53] Furthermore, the group showed that injection of exogenous SDF-1α into the wound acted synergistically with hyperbaric oxygen treatment in mobilizing EPCs into the peripheral circulation. Also related to an impaired response to tissue hypoxia, work by Bosch-Marce *et al* demonstrated in mice that aging leads to a gradual loss-of-function in hypoxia-inducible-factor 1α (HIF-1α). This resulted in decreased progenitor cell mobilization, decreased expression of angiogenic cytokines, and ultimately poor recovery of limb perfusion following ischemic injury. [54] These effects could be reversed, however, by administration of an adenoviral vector that expressed a constitutively active form of HIF-1α. While the process of vasculogenesis is distinct from that of intimal hyperplasia and restenosis, the findings from the wound healing literature will likely be important in achieving a better understanding of progenitor cell mobilization after vascular injury. The forgoing studies reflecting the numerous cytokines, chemokines, and signaling cascades that are involved in progenitor cell mobilization will ultimately need to be considered in the context of pre-existing disease states such as diabetes or advanced age, which may have significant consequences for efficient progenitor cell mobilization and recruitment.

Migration of progenitor cells to the site of injury

Once progenitor cells are in the peripheral circulation, they must be attracted to the site of tissue injury. In addition to playing an essential role in progenitor cell mobilization, the SDF-1α/CXCR4 axis has been reported to be involved in progenitor cell recruitment in numerous injury models including neointimal hyperplasia [55–57]. *In vitro* studies have shown that SDF-1α induces EPC and $CD34^+$ cell migration and $CD34^+$ cell adhesion [58–60]. In the wound healing literature, SDF-1α has been shown to play an important role in the recruitment of EPCs to diabetic wounds in mice. [53] In the mouse wire injury model, Zernecke *et al* demonstrated that blockade of SDF-1α signaling with either a SDF-1α blocking antibody, lentiviral-based local gene transfer of a mutant SDF-1α, or by transplantation of bone marrow cells deficient in CXCR4 resulted in decreased intimal hyperplasia which was associated with decreased bone marrow derived neointimal smooth muscle cells [57]. The authors reported that SDF-1α induced platelet adhesion at the site of injury, and that subsequent release of platelet p-selectin led to progenitor cell adhesion and recruitment. These same mechanistic findings demonstrating an important role of SDF-1α and platelets in progenitor cell recruitment were confirmed by Massberg *et al* who studied this process *in vivo* using real-time video-fluorescence microscopy [61].

On a molecular level, integrins such as VLA-4, LFA-1 and $\alpha_5\beta_1$ have been shown to play essential roles in progenitor cell adhesion [5, 60, 62]. Notably, the $\alpha_5\beta_1$ integrin has been shown to be upregulated by statin therapy [62]. Consequently, administration of simvastatin to rats undergoing carotid balloon injury resulted in accelerated and more complete re-endothelialization due to increased EPC incorporation into the injured artery [62].

Other factors, such as VEGF and its receptor, have also been shown to stimulate progenitor cell migration and recruitment to sites of tissue injury [63–65]. This complex process clearly involves numerous signaling pathways which are just beginning to be understood.

Differentiation of progenitor cells into mature vascular cells

The differentiation of bone marrow derived progenitor cells into vascular smooth muscle cells or smooth muscle-like cells or endothelial cells is the final step in the recruitment of bone marrow cells into the neointimal lesion. This phenomenon has been studied *in vitro* using several different stem and progenitor cell lines. TGF-β, through Smad3 signaling, has been shown to induce neural crest stem cells to differentiate into smooth muscle cells, [66] and PDGF-BB has been demonstrated to induce TR-BME2 cells, a mouse bone marrow derived EPC line, to differentiate into contractile and synthetic SMCs [66, 67]. Both cytokines have been shown to be upregulated after arterial injury. In terms of endothelial cell differentiation, VEGF is the most studied chemokine. Of note, although EPCs are believed to be the major source of ECs, it has also been reported that MSCs cultured in the presence of VEGF can also differentiate into cells with phenotypic and functional features of endothelial cells [68].

It has been proposed that the direct cell/cell contact may be a stimulus of progenitor cell differentiation. Several studies have shown that direct cell-to-cell contact between MSCs and cardiomyocytes or smooth muscle cells results in MSC differentiation into these two cell types [69,70]. Interestingly, it has also been reported that co-culture of MSCs with EPCs drives MSCs to differentiate into endothelial-like cells [32]. Finally, many of the factors that have been found to be important in progenitor cell mobilization and/or migration may also play a role in differentiation. For example, co-culture of eYFP (yellow fluorescent protein) expressing progenitor cells with VSMCs expressing Kit Ligand stimulated differentiation of progenitor cells into smooth muscle cells, as reflected by cells that were double positive for eYFP and α-SMA. Consequently, addition of anti-KitL antibody to stimulated VSMCs expressing KitL resulted in less differentiation [5].

The contribution of different progenitor cells to the arterial response to injury is summarized in Figure 2. Together, these findings demonstrate that although the process of progenitor cell recruitment to sites of injury can be conceptualized in three steps, it is apparent that the steps are closely related in that many signaling molecules play important roles throughout this entire complex process.

Summary

Bone marrow derived progenitor cells represent a new source of smooth muscle cells and endothelial cells that contribute to or modulate intimal hyperplasia after arterial injury. Numerous mouse models have been studied to gain insight into which progenitor cells are important, although the answers are still unclear. Whereas many studies have demonstrated the plasticity of HSCs, it is likely that MSCs also contribute to the smooth muscle cells and perhaps also the endothelial cells that repopulate the injured artery. Identification of the signaling mechanisms that underlie bone marrow progenitor cell recruitment to the neointima is only in its early stages. However several molecules, such as SDF-1α and KitL have emerged as potential targets for molecular therapy. Despite these advances in understanding progenitor cell recruitment, it is also imperative that these observations, made in mouse models of arterial injury, be validated in cases of human disease. If progenitor cells do indeed represent a significant fraction of neointimal cells, then an in-depth understanding of how the cells migrate from the bone marrow to the site of injury is essential for the development of targeted therapies for arterial restenosis.

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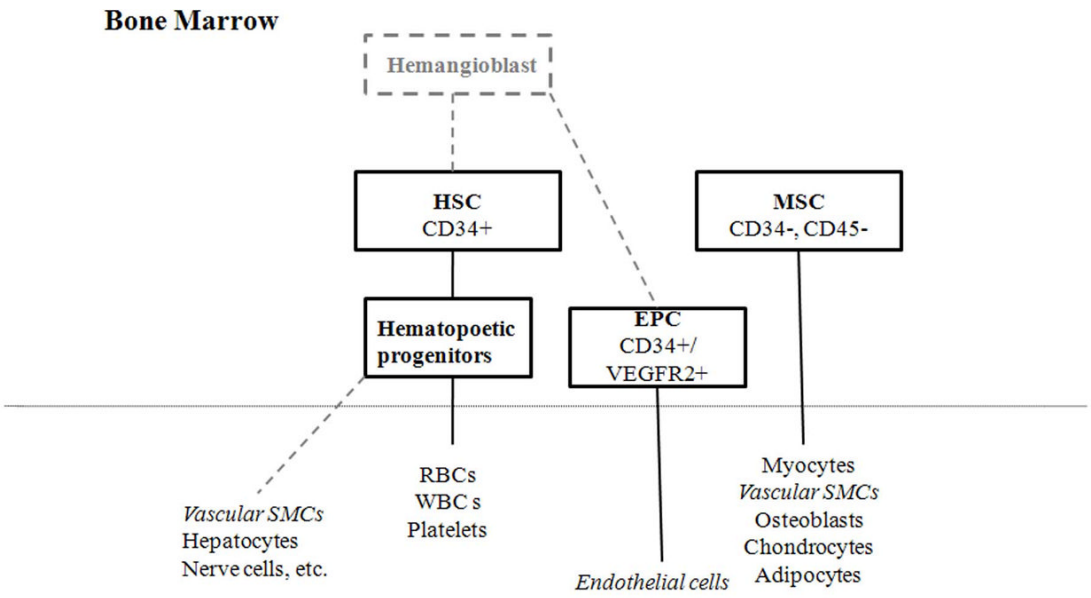

Figure 1.

Schematic of stem cell and progenitor cell classification. The hemangioblast is believed to be an embryonic precursor for HSCs and possibly also EPCs. HSCs differentiate into hematopoietic progenitors and then into the various blood cell lineages, but there is also evidence that HSCs can transdifferentiate into non-hematopoietic cells. MSCs are believed to differentiate into multiple mesenchymal lineages. Dotted grey lines indicate points that are still controversial.

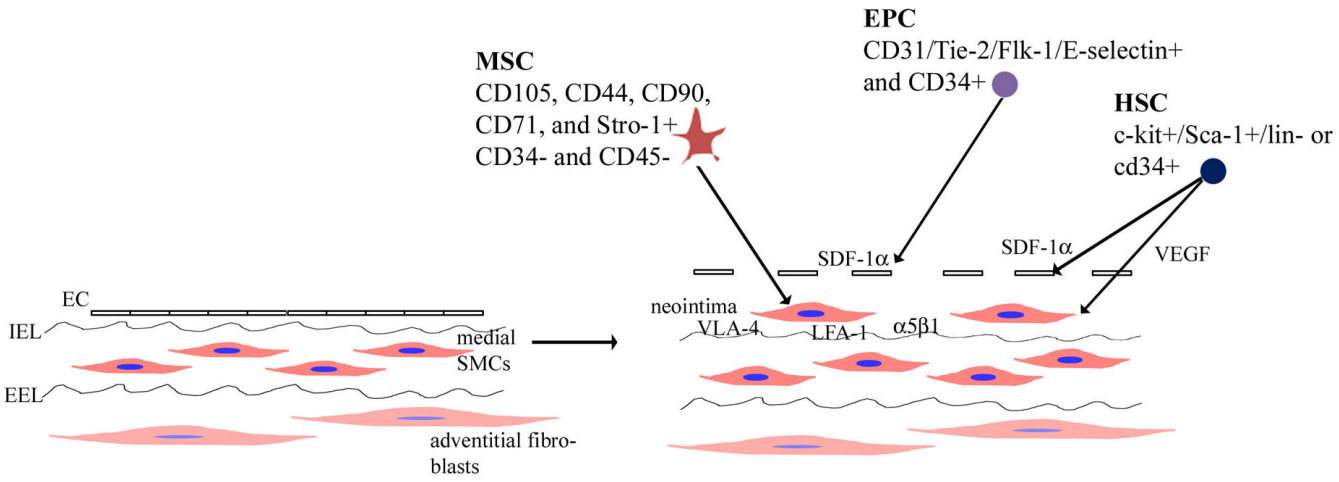

Figure 2.

Table 1

Contribution of bone marrow derived cells to mouse arterial injury models. Since progenitor cells are believed to differentiate into smooth muscle cells or endothelial cells, some studies have also analyzed the percentage of bone marrow derived cells that also express smooth muscle cell markers (SMA+) or endothelial markers, thus signifying differentiation into different types of arterial wall cells.

Experimental model	Determination of cell origin	Time	% BM derived neointimal (NI) or endothelial cells	% BM derived medial cells	publication
Mouse iliac artery wire injury	BMT $^{Male\rightarrow female}$ Y chromosome ISH	4 wks	56% of total cells in NI, 44% of SMA^+ cells	No medial SMA^+ cells	Han C *et al.* 2001[6]
Mouse femoral artery wire injury	$BMT^{ROSA26\rightarrow WT}$ X-gal staining	4 wks	63.0±9.3% of total NI cells	45.9±6.9% total cells	Sata M *et al*. 2002[3]
Mouse carotid wire injury model	Labeling with retrovirus expressing GFP	2 wks	~10% of total endothelial cells	Not reported	Werner *et al.* 2002.[27]
Mouse femoral artery wire injury	$BMT^{eYFP\rightarrow WT}$ IF	4 wks	66±12% of SMA^+ NI cells	Not reported	Wang C-H *et al*. 2006.[5]
Mouse femoral artery wire injury	$BMT^{GFP\rightarrow WT}$ IF	4 wks	20.5±5.7% total NI cells	39.3±3.1% total cells	Tanaka K *et al*. 2003.[4]

ISH= in-situ hybridization, BMT=bone marrow transplant, NI=neointima, SMA=smooth muscle actin, IF=immunofluoresence (to visualize GFP or YFP positive cells)

Table 2

Percentage of GFP^+ cells in the neointima and media of $BMT^{GFP \rightarrow WT}$ mice 4 weeks after injury. $(n=4)^4$

	Wire injury	Perivascular cuff	Carotid artery ligation
Neointima	38.9±5.8%	7.0±2.1%	24.1±5.3%
Media	61.4±5.8	15.1±2.2%	33.1±8.2%

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Table 3

Differential expression of cell surface markers between HSCs, EPCs, and MSCs. Markers may vary between species; mouse markers are shown because it is the most widely studied animal model. One of the most important distinctions between the groups is that while HSCs are CD34+ and CD45+, MSCs are CD34− and CD45−. CD117 is also known as c-kit.

HSC		EPC		MSC	
Human	**Mouse**	**Human**	**Mouse**	**Human**	**Mouse**
CD34+	CD117+	CD34+	CD31+/dim	CD90+	CD90+
CD117+	CD150+	CD31+	VE-cadherin+	CD157+	CD44+
CD90+	Sca-1+	VE-cadherin+		Stro-1+	CD29+
CD243+		VEGFR2+		CD10+	CD49e+
CD133+		E-selectin+			CD105+
CD45+		CD202+			CD71+
		?AC133+			
CD38−	CD48/CD41−	CD14−		CD45−	CD45−
Lineage & minus;	Lineage & minus;	CD15−	CD11b−	CD34−	CD11b−