 

# The Emerging Role of the Bone Marrow-Derived Stem Cells in (Therapeutic) Angiogenesis

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**Key words**

Angiogenesis, bone marrow cells, endothelial cells, multipotent progenitors, growth factors

**Summary**

Proper formation of blood vessels (angiogenesis) is essential for development, reproduction and wound healing. When derailed, angiogenesis contributes to numerous lifethreatening disorders. While research has generally been focusing on the two main vascular cell types (endothelial and smooth muscle cells), recent evidence indicates that bone marrow may also contribute to this process, both in the embryo and the adult. Novel vascular progenitors, even one common to both endothelial and smooth muscle cells, have been identified in the embryo. An exciting observation is that endothelial precursors have also been identified in the adult bone marrow. Transplantation studies revealed that these precursors as well as other bone marrow-derived cells contribute to the growth of endothelium-lined vessels (angiogenesis) as well as the expansion of pre-existing collaterals (arteriogenesis) in ischemic disease. These findings have raised hopes that bone marrow-derived cells might one day become useful for cell-based angiogenic therapy.

## Introduction

Proper formation of blood vessels (angiogenesis) is essential for development, reproduction and wound healing. When derailed, angiogenesis contributes to numerous lifethreatening disorders (1). Blood vessels are primarily composed of two cell types: endothelial cells, lining the inside (*endo*-, inside) and smooth muscle cells, covering the outside. While angiogenesis research has generally been focused on these two vascular cell types, recent evidence indicates that the bone marrow may also contribute to this process, both in the embryo and the adult. An exciting observation is that endothelial and smooth muscle cell precursors have been identified in the adult. Bone marrow-derived cells are able to affect the growth of endothelium-lined vessels (angiogenesis) as well as the expansion of pre-existing collaterals (arteriogenesis). These findings have raised unprecedented hopes that bone marrow-derived cells might one day become useful for cell-based angiogenic therapy. Their role and possible implications for pathology and therapy are discussed in this brief overview.

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## Endothelial Stem Cells in the Embryo

Multipotent progenitors giving rise to distinct differentiated cell types have been found in a variety of systems, including the neural and blood-forming (hematopoietic) systems. Endothelial and smooth muscle cells also arise from their own progenitors. Depending on the location in the embryo, endothelial cells arise from progenitors with a restricted commitment to the endothelial lineage (angioblasts) or from stem cells giving rise to both endothelial and hematopoietic cells (hemangioblasts; Fig. 1) (2, 3). Differentiation of pluripotent embryonic precursor cells into hemangioblastic cells is induced to at least some extent by fibroblast growth factor (FGF) (4). Hemangioblasts undergo their first critical steps of differentiation within the blood islands. Cells at the perimeter of the blood islands give rise to precursors of endothelial cells, while those at the center constitute hematopoietic precursors. The molecular signals determining the fate of the hemangioblast are not fully elucidated. However, several genes have been identified that may play a role in this early event (5). These include *Ets*-1 (6), *Hex* (7), *Vezf*, *Hox* (8, 9), members of the *GATA* family, basic helix-loop-helix *(bHLH)* factors (10, 11), and the *Id*-proteins (12). Early markers common to endothelial and hematopoietic precursors include CD34 and the receptor tyrosine kinase type-2 of vascular endothelial growth factor (VEGFR-2 or KDR/Flk1) (13). In embryonic stem cell-derived embryoid bodies, VEGF stimulates the growth of hemangioblasts and subsequently of lineages, with characteristics of the endothelial lineage including the expression of CD31, VEGFR-2, VEGF receptor-1 or VEGFR-1/Flt1 and Tie-2 (a receptor of the angiopoietins), the capacity to take up acetylated LDL and the presence of cytoplasmic Weibel-Palade bodies (14). In addition, *in vivo* studies in the *Xenopus* embryo and in transgenic mice expressing human VEGF-A have documented a role for VEGF in mediating angioblast migration (15, 16). The role of VEGF may, however, not relate to determining endothelial fate, since endothelial cells still differentiate in embryos lacking VEGF (17). Since VEGFR-2 deficiency blocks differentiation of blood and vascular cells (18), other VEGFR-2 ligands may be essential for endothelial cell fate *in vivo*. VEGF receptor-1 (VEGFR-1; Flt1) has been determined to suppress hemangioblast commitment (19). Other markers of embryonic angioblasts include thrombomodulin, the early mesodermal marker fgf-3, von Willebrand Factor (vWF), GATA-4 and GATA-6 (20).

## Arterial versus Venous Angioblasts

As the yolk sac vasculature begins to form around 7.5 days post coitum (dpc) in the mouse, angioblasts that have migrated to the paraxial mesoderm assemble into aggregates, proliferate and subsequently differentiate to form the dorsal aortae, cardinal veins and the embryonic yolk sac vessels. The specification between vessels conducting blood from or to the heart (arteries and veins, respectively) occurs very early at the angioblast stage. Little is presently known about the molecules

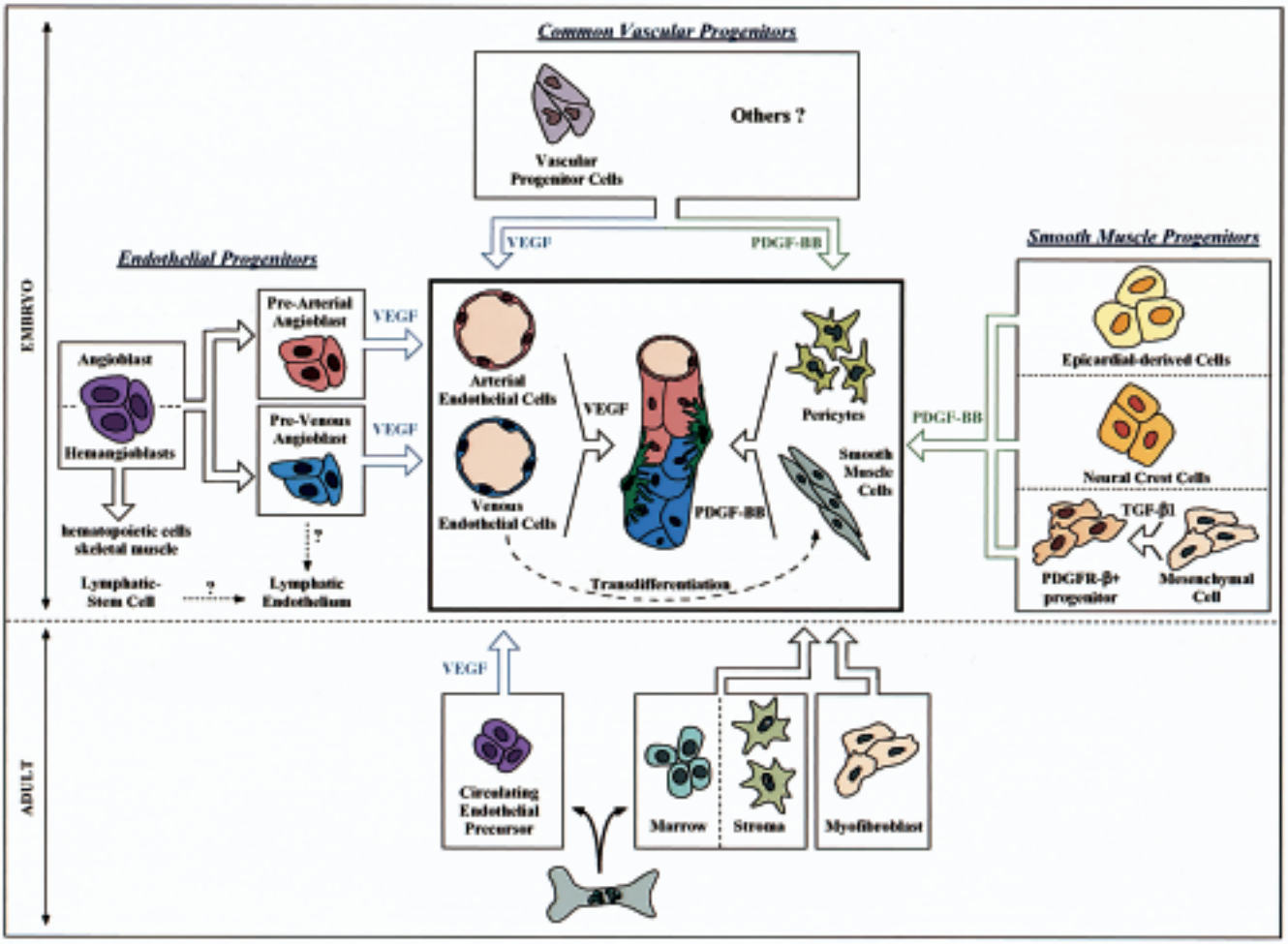


*Fig. 1* A common origin for the two types of blood-vessel cells. Endothelial and smooth muscle cells arise from separate types of precursors. Endothelial cells arise from precursors called angioblasts or hemangioblasts in the embryo, or from circulating endothelial progenitors in the adult. Angioblasts give rise to arterial and venous lineages. Smooth muscle cells and pericytes, in contrast, can form from a variety of progenitors. These include mesenchymal cells, neural crest cells, and progenitors in the epicardium in the embryo. Progenitors in the bone marrow and its stroma, and mesenchymal myofibroblasts also give rise to smooth muscle cells. A new common vascular progenitor cell that gives rise to both types of blood-vessel cell has been recently identified. Vascular endothelial growth factor (VEGF) promotes the development of endothelial cells from this precursor. TGF-β1 has been involved in differentiation of mesenchymal cells to progenitors, which express the receptor for PDGF-BB. The latter stimulates their development into smooth muscle cells and pericytes and is responsible for their recruitment around nascent vessels. Adapted from (5)

implicated in the arterial-venous specification. Genetic studies in the zebrafish have identified the basic helix-loop-helix transcription factor gridlock A as a possible candidate for this process – with gridlock A favoring differentiation of pre-arterial at the expense of pre-venous angioblasts (Fig. 1) (21). Notch-derived signals, which are often involved in cell fate determination via lateral specification and inductive signaling between distinct cell types, might also be implicated. Recent evidence indicates that Delta4, a Notch ligand, is expressed in arterial endothelium (22) and that defective Notch signaling caused vascular remodeling defects (23, 24). The hairy-related bHLH factor HeyL, an effector of Notch, is expressed in smooth muscle of all arteries, overlapping with that of Notch3. Mutations of *Notch3* underlie the CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy) vascular disorder (25).

There is mounting evidence to indicate that members of the large ephrin family may also play a role in arterial-venous specification. Ephrins are ligands for their corresponding Eph receptors that form a family of at least 14 receptor tyrosine kinases (26, 27). Ephrins must be membrane bound to activate their receptors. Ephrin A1 and ephrin A2 are angiogenic, with specificity based on the vascular bed (28). Ephrins B1 and B2 induce sprouting. Ephrin B2 is restricted to arteries, whereas its receptor, EphB4 is found in veins – not in arteries (29). Inactivation of the *ephrin B2* gene in mice results in normal vasculogenesis but abnormal angiogenesis, the latter with disrupted remodeling of both arteries and veins into large and small branches, and diminished vessel maturation with decreased association of peri-endothelium (30, 31). Complex ligand-receptor bidirectional signaling interactions via ephrins and ephrin receptors have been described, with responses dependent on a variety of factors, including phosphorylation, multimerization, and the presence of adaptor proteins, such asGrb2, Grb10 and Nck. A novel cytoplasmic tyrosine kinase gene, bone marrow tyrosine kinase *(Bmx)*, was identified in arterial endothelium (32). Since its loss did not affect physiological growth of arteries in knockout mice (K. Alitalo and P. Carmeliet, unpublished), the function of *Bmx* needs to be further defined. Initial observations from our own laboratory reveal that selective loss of the $VEGF_{164}$ isoform in mice prevents normal arterial development in the retina (unpublished). To what extent vascular growth factors selectively affect arterial or venous growth in the adult remains outstanding. Future studies will be required to unravel the differentiation characteristics of veins, arteries and capillaries (33).

### Tissue-specific Differentiation of Endothelial Cells

With maturation of the vascular network, local physiological requirements must be met. To this end, endothelial cells acquire highly specialized characteristics to provide the functional needs within specific tissues and organs. For example, development of the blood-brain barrier requires interactions between astroglial cells which express glial fibrillary acidic protein, pericytes and adequate angiotensinogen levels (34). The tight junctional complex between endothelial cells consists of numerous integral membrane and cytosolic proteins from, for example, the families of cadherins, occludins, claudins, and membrane-associated guanylate kinase homologous proteins (35, 36). The plasma membranes of those endothelial cells reaching confluence necessarily undergo major structural and functional modifications, crucial for the regulation of vascular permeability. Other proteins, such as 7H6, cingulin and JAM participate in monocyte transmigration and regulation of permeability (36, 37). In contrast, endothelial cells in endocrine glands lack the tight junctions of the blood-brain barrier. Rather, the endothelium is discontinuous and fenestrated, allowing high-volume molecular and ion transport. Overall, the factors which regulate acquisition of specific endothelial properties are largely unknown. However, it appears that the host environment, in concert with VEGF, plays a major role (38, 39). Whether organ-restricted endothelial progenitors exist, remains largely unknown. Endothelial cells of the lymphatic vessels, which absorb the fluid leaking out of blood vessels, sprout from venous endothelium, but we do not know whether they arise from a lymphatic stem cell (Fig. 1).

Bone marrow endothelial cells (BMEC) are an essential component of the bone marrow microenvironment and form a unique type of endothelium that supports hematopoiesis and cell trafficking from (mobilization) and to (homing) the bone marrow. To allow selective migration of hematopoietic stem and progenitor cells through the bone marrow-blood barrier, BMEC constitutively express a set of adhesion molecules, like E-selectin (40) and vascular adhesion molecule-1 (VCAM-1) (41). Down- or upregulation of these adhesion molecules may contribute to the regulation of transendothelial migration (42, 43). Different hematopoietic cells have been shown to produce angiogenic factors, like VEGF, which can further influence cell trafficking by affecting endothelial fenestration and by modulating expression of adhesion molecules on BMEC (42). Recently it was shown that fenestrated capillaries in the bone marrow as well as cultured human BMEC express VEGF receptor-3 (VEGFR-3/Flt-4) indicating a role for its ligands VEGF-C and VEGF-D in regulating cell mobilization and homing (44, 45). The specific effects of the different VEGF-family members on mobilization of hematopoietic and endothelial cell precursors need to be further explored.

Endothelial cells also acquire specific characteristics in tumors. Ultrastructurally, tumor vessels are abnormal: their walls have numerous "openings" (endothelial fenestrae, vesicles, and transcellular holes), widened inter-endothelial junctions, and a discontinuous or absent basement membrane. The endothelial cells are abnormal in shape, growing on top of each other and projecting into the lumen. These defects make tumor vessels leaky (46-48). However, there is heterogeneity in leakiness over space and time and in response to treatment (49). Vascular permeability and angiogenesis depend on the type of tumor and the host organ where the tumor is growing (50), in part because each organ has different stromal cells which produce different pro- and anti-angiogenic molecules (51, 52). Low permeability tumors may over-express angiopoietin-1 (Ang1) and/or under-express VEGF or its homologue placental growth factor (PlGF). Conversely, those with high permeability may lack Ang1 or over-express its antagonist Ang2 (53). Tumor vessels express surface proteins ("vascular zip codes") which are absent or barely detectable in mature vessels (54, 55). *In vivo* selection of phage display libraries has recently yielded peptides (e. g., RGD, NGR) which preferentially recognize vessels in subcutaneous tumors in mice (56).

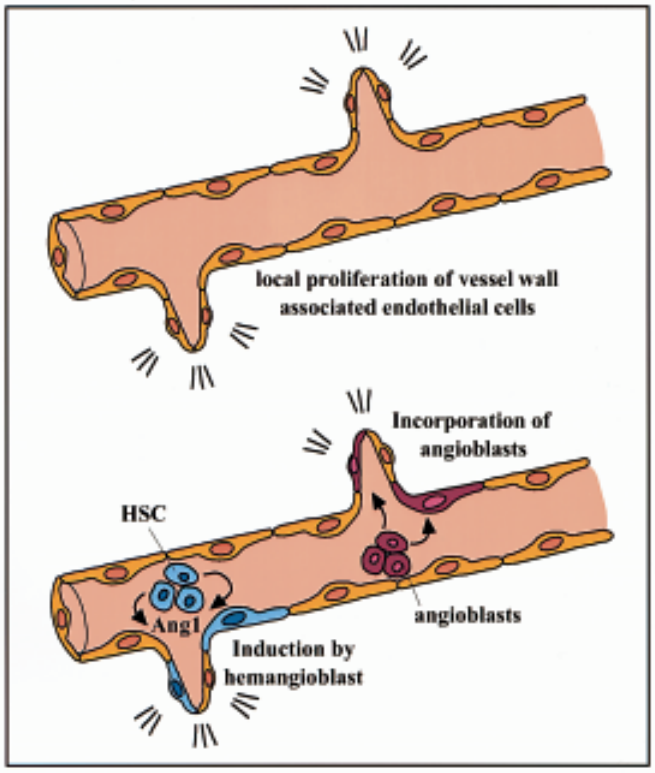


*Fig. 2* New angiogenic sprouts may form as a result of local proliferation of endothelial cells, associated with the vessel wall (upper panel), or of mobilization and incorporation of EPCs into the growing sprouts (lower panel; right). In addition, hematopoietic or endothelial precursors may create a micromilieu at the growing tip of angiogenic sprouts by releasing angiogenic growth factors (lower panel; left)

Recent findings suggest that tumor vessels may be lined not only by endothelial cells, but by tumor cells themselves which have attained "vasculogenic" properties or a mosaic of cancer and endothelial cells (57). For example, 15% of vessels in xenografted and spontaneous human colon carcinomas are mosaic in nature (58). So-called "vasculogenic mimicry", which implies *de novo* generation of vasculature without participation of endothelial cells and independent of angiogenesis, would have a major impact on therapy designed to interfere with new vessel growth associated with tumorigenesis (59).

### Hematopoietic Stem Cells Affect Embryonic Vascular Development

Hematopoietic and endothelial stem cells not only share a common origin, the former can also stimulate the assembly of endothelial cells into nascent blood vessels in the embryo. Indeed, hematopoietic stem cells were found at sites of active vascular expansion. By producing Ang1, these cells stimulated endothelial growth in the embryo (Fig. 2) (60). Whether hematopoietic stem cells also contribute to pathological angiogenesis in the adult remains outstanding.

### Smooth Muscle Progenitors in Development

Several cell types surround endothelial channels: layers of smooth muscle cells around large vessels in proximal parts of the vasculature and single pericytes around smaller distal vessels (Fig. 1). Similar to vascular smooth muscle cells, pericytes covering arterioles, venules and capillaries serve multiple functions, modulating blood flow and vascular permeability, regulating growth of blood vessels, and providing signals to endothelium and matrix via secreted and cellular molecules (61). These mural cells have a complex origin, depending on their location in the embryo (62). The first smooth muscle cells around endothelial tubes in the embryo transdifferentiate from the endothelium (62). Endothelial cells also transform to smooth muscle-like myofibroblasts in the prospective cardiac valves – a process involving signaling by transforming growth factor-β3 (TGF-β3) (63). TGF-β1, another family member, has been implicated in the differentiation of a mesenchymal stem cell to a progenitor, that expresses platelet-derived growth factor receptor-β (PDGFR-β) (64, 65). By releasing platelet-derived growth factor-BB (PDGF-BB), endothelial cells stimulate subsequent growth and differentiation of this precursor. Pericytes and smooth muscle cells of the coronary vessels are derived from a putative progenitor that infiltrates the heart from its external (epicardial) layers (62). Coronary vein smooth muscle cells are derived from the atrial myocardium, while those of the coronary arteries come from the epicardial layer (66). Differentiation of coronary smooth muscle cells from proepicardial cells involves serum response factor (SRF), a member of the MADS box family of DNA binding proteins (67). Cardiac neural crest cells are the source of smooth muscle cells of the large thoracic blood vessels, a not infrequent site of congenital malformations (68). Whether smooth muscle cells and pericytes in arteries, veins and lymphatic vessels or even within the inner andmedial layer of an artery arise from distinct progenitors remains unknown.

### Common Vascular Progenitor in Development

Yamashita et al. (69) recently discovered an embryonic common vascular progenitor, that differentiates both into endothelial and smooth muscle cells (Fig. 1). The smooth muscle cells arising from these progenitors were not simply transdifferentiated endothelial cells expressing the atypical smooth muscle alpha-actin marker. Instead, they expressed an entire set of smooth muscle markers and surrounded endothelial channels *in vivo*. This common vascular progenitor resembles somehow the putative common precursor of the endothelial cells that line the inner surface of the heart (the endocardium) and their surrounding cardiac muscle fibers (70). Like hemangioblasts, vascular progenitors express VEGFR-2 (69), raising the question of whether both cell types arise from a multipotent stem cell. The vascular progenitors differentiated to endothelial cells in response to VEGF, whereas they developed into smooth muscle cells in response to platelet-derived growth factor-BB (PDGF-BB) (69). It is possible that PDGF-BB is a determinant of smooth muscle cell fate, but PDGFR-β-positive progenitors still develop in the absence of PDGF-BB *in vivo* (64) and *in vitro* (69). Thus, PDGF-BB may favor the selection and growth of PDGFR-β-expressing progenitors. Such a hypothesis is consistent with a model whereby PDGF-BB stimulates PDGFR-β-expressing progenitors to migrate along pre-existing endothelial channels and to divide during arterial enlargement (64). A common vascular progenitor could contribute to the formation of naked endothelial capillaries (angiogenesis) and muscle-coated vessels (arteriogenesis).

### Endothelial Progenitor Cells in Pathological Angiogenesis

Sprouting of new vessels from pre-existing vessels in the adult has generally been considered to rely on proliferation and migration of local vessel wall-associated endothelial cells in response to angiogenic stimuli, generated at the site of active angiogenesis ("local endothelial growth"; Fig. 2). Vasculogenesis, which refers to the initial events in vascular growth in which endothelial cell precursors migrate to discrete locations, differentiate *in situ* and assemble into endothelial channels, was originally believed to be restricted to embryonic development. However, recent studies indicate that endothelial progenitor cells (EPCs) also circulate postnatally in the peripheral blood and may be recruited for *in situ* vessel growth (71-74) (Fig. 2, 3). In addition, EPCs have been shown to be involved in re-endothelialization of implants (75-78).

EPCs were initially isolated on the basis of their expression of VEGFR-2 and CD34, antigens shared by both the angioblast and the hematopoietic progenitor. These EPCs were subsequently shown to express AC133, an orphan receptor which is specifically expressed on EPCs but whose expression is lost once they differentiate in more mature endothelial cells (75, 79). EPCs express endothelial-specific markers VE-cadherin and E-selectin (75). VEGF, stem cell growth factor (SCGF), bFGF, insulin-like growth factor-1 and other cytokines differentiate these progenitor cells to mature endothelial cells *in vitro* (76, 79). Most CD34/VEGFR-2-positive cells also express the chemokine receptor CXCR4 and migrate in response to stromal-derived factor (SDF)-1 or VEGF (75). Unlike shed cells, circulating bone marrow-derived angioblasts in the adult have a high proliferation rate (80).

Transplantation studies have revealed that these EPCs can be incorporated into sites of active angiogenesis in ischemic hindlimbs and myocardium, injured corneas and tumor vasculature (73, 79, 81) as well as during repair of denuded endothelial cells (82). How angioblasts know where and when to initiate vasculogenesis is largely a mystery. In response to signals from cancer cells or healing wounds, quiescent endothelial progenitors are recruited from the peripheral blood and bone marrow (73, 74, 83, 84). Ischemia or vascular trauma may be a significant stimulus for mobilization of EPCs from the bone marrow, as evidenced by the increased recruitment of EPCs in corneal angiogenesis when hindlimb ischemia was present in mice (74, 84), as well as by the rapid EPC mobilization in patients with vascular trauma (85). A variety of growth factors including granulocyte macrophage-colony stimulating factor (GM-CSF), VEGF and Ang1 have been demonstrated to recruit bone-marrow derived angioblasts to sites of neovascularization postnatally (71, 75, 84).

VEGF likely plays a significant role in this process, since it stimulates EPC mobilization *in vivo*, is induced in hemangioblasts upon cytokine stimulation (86), and is chemoattractive for angioblasts (15, 86), likely via expression of VEGFR-2 (75). In theory, VEGF might enhance transendothelial migration of EPCs by making the blood bone-marrow barrier more permeable (VEGF is a well-known permeability factor [48]), by expanding the bone marrow vasculature (87) or by modulating expression of adhesion molecules (E-selectin) on bone marrow endothelial cells (42). Furthermore, VEGF induces the release of hematopoietic growth factors (GM-CSF) by bone marrow endothelial cells and increases the *in vitro* SDF-1 driven transendothelial progenitor cell migration, possibly due to increased endothelial fenestration (86). Thus, release of VEGF by progenitor cells may result in a paracrine loop supporting proliferation of both endothelium and progenitors and may facilitate transendothelial migration during cytokine-induced

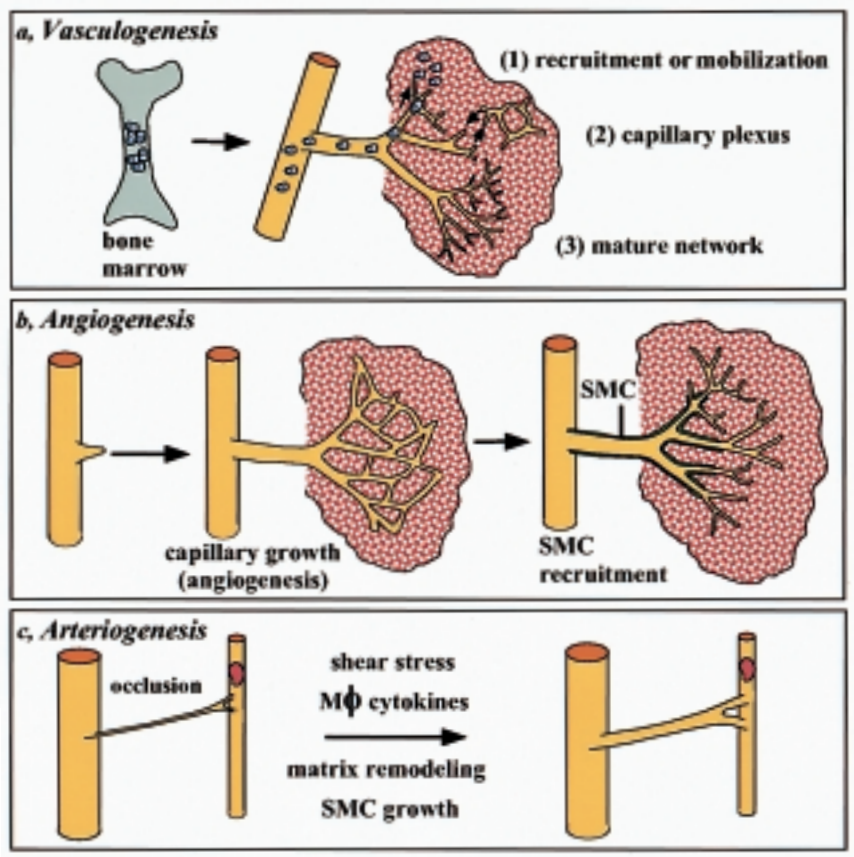


*Fig. 3* Pathological vascular growth in the adult may occur via angiogenesis (sprouting), arteriogenesis (collateral growth) or vasculogenesis (mobilization of EPCs)

progenitor cell mobilization. Placental growth factor (PlGF), a homologue of VEGF, could also play a role in this process, as transplantation of wild type bone marrow partially restored the impaired neovascularization of matrigel implants in PlGF deficient mice (unpublished results). These studies indicate that EPCs might contribute to adult vascular growth by direct incorporation into the expanding vasculature. It remains to be determined to what extent these progenitors, like adult neural stem cells, also play a role in tissue repair by activating endogenous cells to provide self-repair, for instance by supplying trophic factor support (Fig. 2). The contribution of EPCs to pathological angiogenesis, relative to the local growth of vessel wall-associated endothelial cells in the angiogenic sprouts is largely unknown, but EPCs have been estimated to contribute to as little as a few to as much as 25% of the newly formed vessels. Their contribution is likely to depend on the tissue and pathological disorder.

### Role of Other Bone Marrow-derived Cells in Pathological Vessel Growth

Blood-born cells have been implicated in angiogenesis during wound healing and cancer. Upon tissue injury, activated platelets initially form a hemostatic plug to occlude the vessel wall defects. Subsequently, a granulation reaction takes place to restore the integrity of the injured vessel wall and the tissue: neutrophils and, subsequently, macrophages infiltrate the wound to remove the debris, whereas endothelial cells re-endothelialize denuded vessels and form numerous new capillaries to deliver oxygen and nutrients to the hypoxic wound. Finally, fibroblasts form a collagen-rich scar. At this stage, the excessive amount of vessels is pruned and the immature capillary plexus is remodeled into a more mature and stable vascular network. Most wound cells have been implicated in angiogenesis during wound healing via release of positive angiogenic regulators (88, 89). Not surprisingly, however, they also produce angiogenesis inhibitors, likely involved in controlling excessive angiogenesis and possibly also responsible for the regression of vessels during scarring (89).

Platelets secrete numerous positive and negative regulators of angiogenesis (90, 91). Angiogenic stimulators include VEGF, its homologue VEGF-C, bFGF, Ang1, hepatocyte growth factor (HGF), epidermal growth factor (EGF), PDGF, etc. Platelet-derived negative regulators of angiogenesis include platelet factor-4, thrombospondin-1, TGF-β1, etc. The plasminogen activator inhibitor-1 (PAI-1) can inhibit endothelial cell migration *in vitro*, but appears to be required for proper vessel formation *in vivo*, presumably by preventing excessive extracellular matrix breakdown, stabilizing the nascent blood vessels (92). A number of other angiogenesis inhibitors, often proteolytically cleaved fragments from hemostatic or fibrinolytic proteins, are generated during clotting. They include fragments of prothrombin, anti-thrombin III or plasminogen (yielding angiostatin) (90). Platelets may also modulate tumor angiogenesis when they become trapped and adhere to the subendothelial matrix in the tumor vessels with a markedly reduced blood flow and hyperpermeability. Activation of the coagulation cascade and platelet aggregation may explain why hemostatic and bleeding disorders often occur in patients with cancer.

Neutrophils, monocytes, macrophages, mast cells and other leukocytes release a myriad of angiogenic factors including VEGF, Ang1, bFGF, TGF-β1, PDGF, tumor necrosis factor-alpha (TNF-α), HGF, insulin-like growth factor-1 (IGF-1), monocyte chemoattractant protein-1 (MCP-1), among many others (88, 89, 93). Some of these factors attract wound cells, which in turn release additional angiogenic factors (94, 95). Blood cells also contain proteinases that degrade

anatomical barriers for migrating vascular cells (96), and activate or liberate some of these growth factors from the extracellular matrix (95, 97). In addition, they can expose cryptic adhesion sites, hidden in non-proteolyzed matrix components. Granulated metrial gland cells, belonging to the natural killer cell lineage, and macrophages in the uterine environment during pregnancy modulate the maternal uterine vasculature (98), in part by producing VEGF (99). VEGF may facilitate recognition of angiogenic vessels in growing tumors by activated natural killer cells, whereas bFGF may provide such vessels with a mechanism which protects them from cytotoxic lymphocytes (100).

Connective tissue mast cells are topographically associated with small vessels. Additional mast cells become recruited to angiogenic sites in arthritis, wound healing, ovulation, myocardium and tumors (101). Tumor angiogenesis in mast cell deficient W/Wv mice is impaired and can be restored upon bone-marrow repair of the mast-cell deficiency (102). Mast cells may regulate angiogenesis directly via release of histamine or TNF-α, or indirectly via affecting other wound cells (101). Recent studies indicate that mast cells infiltrate hyperplasias, dysplasias, and invasive fronts of skin carcinomas, but not the core of solid tumors, where they degranulate in close apposition to capillaries and epithelial basement membranes, releasing mast-cell-specific serine proteases MCP-4 (chymase) and MCP-6 (tryptase) (95). By activating progelatinase B (matrix metalloproteinase-9), which releases sequestered VEGF from extracellular matrix stores (103), MCP-4 induces hyperplastic skin to become angiogenic. Notably, premalignant angiogenesis is ablated in a mast-cell-deficient mouse model of skin carcinogenesis (95).

Monocytes/macrophages have been implicated in the growth of pre-existing collateral arterioles after occlusion of a supply artery in the myocardium and peripheral limbs (94, 104) (Fig. 3). This process has been termed "adaptive arteriogenesis" to denote the distinct cellular and molecular mechanisms from those in true angiogenesis (capillary growth). As a result of the increased shear stress in collaterals, endothelial cells express chemokines (MCP-1) and adhesion molecules like intercellular adhesion molecule-1 (ICAM-1). The recruited monocytes infiltrate and proteolytically remodel the vessel wall (94). Activated endothelial cells then upregulate bFGF, PDGF-B and TGF-β1, which stimulate smooth muscle cell growth and vessel enlargement. It is possible that flow provides the necessary survival signals for maintenance of collaterals. Adaptive arteriogenesis finally results in functional and structurally normal arteries, which ameliorate the detrimental effects of vessel obstruction (104). These vessels may be superior to newly formed capillaries (formed by angiogenesis), because they are able to sustain proper circulation and to adapt to changes in physiological demands of blood supply. Therefore, we should critically consider whether therapeutic stimulation of new blood vessels in ischemic tissues should be aimed at improving angiogenesis or, perhaps preferably, arteriogenesis.

### Bone Marrow Cells for Therapeutic Angiogenesis and Arteriogenesis?

Since bone marrow is a natural source of multiple angiogenic growth factors including aFGF, bFGF and VEGF, and because of the essential involvement of several bone marrow-derived cells (including vascular progenitors) in angiogenesis, several groups have investigated whether transplantation of the entire bone marrow or specific progenitor-enriched cell populations, or their direct implantation into ischemic tissues would enhance tissue vascularization and function. Such studies have revealed that transplantation of bone marrow cells stimulated angiogenesis in a rat cornea model (105). In a rat ischemic heart model, implantation of autologous bone marrow cell induced angiogenesis in the ischemic myocardium, possibly as a result of elevation of the levels of IL-1β and cytokine-induced neutrophil chemoattractant (CINC) (106). Furthermore, autologous bone marrow cells transplanted into ventricular scar tissue differentiated into cardiomyocytes, induced angiogenesis in the scar and restored myocardial function (107).

Studies from our own laboratory using a transgenic mouse model of myocardial infarction revealed the essential role of bone marrow-derived cells in the revascularization of the ischemic myocardium (96, 97). Loss of the urokinase-type plasminogen activator (u-PA) aborted healing of myocardial infarcts after coronary ligation, with negligible angiogenesis occurring in the infarct. u-PA deficient mice were even resistant to treatment with VEGF, which failed to stimulate myocardial angiogenesis. In wild type mice, a close temporo-spatial relationship was observed between infiltration of leukocytes and infarct revascularization. The essential role of these inflammatory leukocytes was further underscored by selective elimination of neutrophils, which prevented revascularization. In addition, infiltration of neutrophils and monocytes was prevented in u-PA deficient mice, coincident with a reduction in myocardial angiogenesis. Notably, transplantation of wild type bone marrow in u-PA deficient mice completely restored infiltration of myocardial infarcts by inflammatory cells and rescued infarct revascularization. Although neutrophils and monocytes appeared to be involved, we cannot exclude that bone marrow-derived EPCs or other cells from the bone marrow also contributed to infarct revascularization.

Initial observations suggest that EPCs might have a therapeutic potential for improving perfusion in ischemic tissues. Indeed, human endothelial progenitor cells (hEPCs), isolated from healthy adult human subjects and *ex vivo* expanded in the presence of the growth factors VEGF, bFGF, insulin-like growth factor (IGF) and epidermal growth factor (EGF) for 7-10 days, contributed to neovascularization in athymic nude mice with hindlimb ischemia (108). Blood flow recovery and capillary density in the ischemic hindlimb were markedly improved, and the rate of limb loss was significantly reduced (108). Since VEGF and GM-CSF treatment enhanced the recruitment of EPCs in animal models (71, 84), as well as in humans (72, 109), controlled use of these cytokines could be considered to boost the mobilization of EPCs from the bone marrow prior to their isolation from the peripheral blood. Indeed, gene transfer of VEGF in patients with limb ischemia or inoperable coronary disease augmented a population of circulating EPCs, expressing endothelial lineage markers VEGFR-2, VE-cadherin, CD34, $\alpha_v\beta_3$, and E-selectin (72, 109). Since EPCs isolated from human peripheral blood mononuclear cells harvested from healthy adult human subjects may be *ex vivo* expanded upon incubation with endothelial mitogens, including VEGF, bFGF, IGF, and EGF, for 7-10 days to yield almost a 100-fold expansion of cells expressing the EC-specific antigens KDR, CD31 and VE-cadherin, cell transplantation might complement current strategies of therapeutic angiogenesis, based on the administration of recombinant growth factors or on gene transfer, for patients in whom endothelial cells fail to sufficiently respond to these treatments. Since animal studies have demonstrated that atherosclerosis, diabetes, aging, etc. impair the angiogenic response, EPC-transplantation might have a significant potential in the future. In support of this, transplantation of a progenitor-enriched cell population significantly enhanced blood-flow restoration in ischemic hindlimbs of diabetic mice (74).

Taken together, bone marrow-derived cells or progenitors might expand the armementarium of therapeutic angiogenesis, in particular

during senescence or disease when the reparative growth potential of vessel-associated vascular cells becomes limited. Vascular progenitors could be also useful to treat ischemic heart disease, as this requires growth of both endothelial and smooth muscle cells. Understanding the signals that mediate their growth and differentiation and identifying markers specific to these progenitors will provide novel tools to explore these future avenues.

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