Review

Cell Trafficking of Endothelial Progenitor Cells in Tumor Progression

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Abstract

Blood vessel formation plays an essential role in many physiologic and pathologic processes, including normal tissue growth and healing, as well as tumor progression. Endothelial progenitor cells (EPC) are a subtype of stem cells with high proliferative potential that are capable of differentiating into mature endothelial cells, thus contributing to neovascularization in tumors. In response to tumor-secreted cytokines, EPCs mobilize from the bone marrow to the peripheral blood, home to the tumor site, and differentiate to mature endothelial cells and secrete proangiogenic factors to facilitate vascularization of tumors. In this review, we summarize the expression of surface markers, cytokines, receptors, adhesion molecules, proteases, and cell signaling mechanisms involved in the different steps (mobilization, homing, and differentiation) of EPC trafficking from the bone marrow to the tumor site. Understanding the biologic mechanisms of EPC cell trafficking opens a window for new therapeutic targets in cancer. Clin Cancer Res; 19(13); 3360–8. ©2013 AACR.

Introduction

Blood vessel formation plays an essential role in many physiologic and pathologic processes, including normal tissue growth and healing, as well as tumor progression. Vasculogenesis is the process by which blood vessels are formed de novo, and angiogenesis is the expansion and remodeling of the existing blood vessel network. Both vasculogenesis and angiogenesis occur during embryonic development. In adult life, revascularization is essential for the survival of growing, injured, and ischemic tissue (1). For many years, it was believed that the sole mechanism responsible for the development of new vascular networks was angiogenesis (2). The process of angiogenesis is regulated by a balance between multiple endogenous pro- and antiangiogenic factors. These include modifications of the fibroblast growth factor and VEGF families. Modifications in these factors and in balance can lead to cancer progression; the imbalance of pro- and antiangiogenic factors activates an "angiogenic switch" (3). Various cell types have been identified as participants in the angiogenic switch, including endothelial cells, vascular smooth muscle cells, stromal cells, and parenchymal cells (4).

In 1971, Folkman and colleagues hypothesized that tumor growth was angiogenesis dependent and emphasized that future possibility of inhibition of angiogenesis could be useful as therapy against cancer (5). Cancer progression is largely dependent on tumor vascularity, and new vessel formation ensures adequate supply of nutrients, oxygen, and growth factors to the growing tumor and facilitates tumor dissemination (6). Three patterns of blood supply to tumors have been proposed: vasculogenic mimicry, mosaic vessels, and endothelium-dependent vessels (7). One important way by which angiogenesis facilitates tumor metastasis is by providing a route of exit for tumor cells to leave the primary site and enter the bloodstream during metastasis (8). Besides sprouting from preexisting endothelial cells, tumors use other methods of vessel growth, where neoangiogenesis is facilitated through recruitment of endothelial cells to the tumor vascular bed (9). Vasculogenesis occurs when circulating endothelial precursors are recruited in response to factors secreted by tumor cells, resulting in the generation of new vessels in the tumor (7).

Mobilization of the endothelial progenitor can occur in response to oxygenation in tissues to promote angiogenesis, for example in response to tumor growth and hypoxia and in response to tissue ischemia after myocardial infarction. Although the first is considered a negative phenomenon, the second is considered a positive one. In this review, we focus on the role of endothelial progenitor cell (EPC) trafficking in tumor progression.

Definition of EPCs

EPCs are a subtype of stem cells with high proliferative potential that are capable of differentiating into mature endothelial cells and contributing to neovascularization...
EPCs are found mainly in the bone marrow in adults, but they are also detected in the peripheral blood; moreover, they are detected in fetal liver and in umbilical cord blood (10). The first description of isolation of putative progenitor endothelial cells for angiogenesis was published in 1997 (11). Further studies showed that tumor neovascularization occurs through bone marrow–derived EPCs (12). Essentially, two types of cells compromise the putative circulating EPC definition: proangiogenic hematopoietic cells (early EPCs) and outgrowth endothelial cells (late EPCs; refs. 13, 14). With conflicting results reported in the field, the definition, identification, and characterization of EPCs are still not clear. Figure 1 summarizes the expression of the different markers during the trafficking and differentiation of the EPCs to endothelial cells. EPCs are generally identified by expression of several surface markers that characterize the functionality of the EPCs, including CD133 (an early hematopoietic stem cell marker), CD34 (a progenitor marker), and the VEGF receptor-2 (VEGFR-2, an endothelial marker, also termed kinase insert domain receptor or Flk-1; refs. 10, 11). EPCs from different sources express different markers. Bone marrow–derived EPCs are immature cells expressing CD133+/CD34+/VEGFR-2+/VE-cadherin+ (vascular endothelial cadherin), whereas EPCs isolated from peripheral blood are more mature and express a variety of markers that are typical for the endothelial lineage, including platelet endothelial cell adhesion molecule-1 (CD31), melanoma cell adhesion molecule (CD146), VE-cadherin (CD144), endothelial nitric oxide synthase (eNOS), and von Willebrand factor (vWF; refs. 15, 16). While early peripheral blood–derived EPCs express CD133+/CD34+/VEGFR-2+/VE-cadherin+/eNOS+/vWF+, late peripheral blood–derived EPCs express CD133+/CD34+/VEGFR-2+/CD31+/CD146+/VE-cadherin+/eNOS+/vWF+ (16). Mature endothelial cells are terminally differentiated cells with a low proliferative potential expressing CD133+/CD34−/VEGFR-2+/CD31+/CD146+/VE-cadherin+/eNOS−/vWF− (15, 17).

Role of EPCs in Tumor Angiogenesis

Physiologic and pathologic conditions and mediators have been described to affect the number of EPCs and their functions. Estrogens, erythropoietin (Epo), VEGF, stromal cell–derived factor-1 (SDF-1), and physical exercise were shown to increase the number of circulating EPCs; diabetes, smoking habit, and vascular diseases decrease the number of circulating EPCs (15, 17). In addition, physical exercise was shown to enhance circulating EPC levels. Although the mechanism is not fully understood, it has been shown that upregulation of EPCs by exercise is dependent, at least in part, on endothelial nitric oxide and VEGF (18).

Increased numbers of circulating EPCs were observed in several cancers, including gliomas, non–small lung cancer, myeloid leukemia, hepatocellular carcinoma, colorectal cancer, lymphoma, and breast cancer as well as in atherosclerosis (15, 17). EPCs play an important role in the growth and angiogenesis of tumors at both early and late stages (19). Different subpopulations of EPCs play different roles in the angiogenesis process in which early EPCs augment angiogenesis, whereas late EPCs directly participate in tubulogenesis (20).

EPCs participate in different functions in tumor angiogenesis and vasculogenesis, as well as in the maintenance of vascular homeostasis (1, 21). It is hypothesized that once
recruited to tumor sites, EPCs have a dual role in tumor angiogenesis: to regulate the angiogenic process via paracrine secretion of proangiogenic growth factors (21) and to provide structural function and direct luminal incorporation into sprouting nascent vessels (22, 23). The generation of a lethal tumor mass requires both tumor cell proliferation and angiogenesis (24); tumor cell proliferation alone, in the absence of angiogenesis, can give rise to dormant microscopic tumors but remain harmless to the host (6). Figure 2 summarizes the dual role of EPCs in tumor angiogenesis. There is evidence suggesting that a hypoxic microenvironment within a tumor may promote the development of tumor-derived endothelial cells in glioblastoma. These tumor-derived endothelial cells are distinguishable from conventional endothelial cells due to expression of some tumor-specific markers (25).

DNA-binding protein inhibitors (Id proteins) in EPCs were shown to be critical for tumor angiogenesis; these proteins interact with basic helix-loop-helix transcription factors to regulate differentiation and cell-cycle progression (26). Knocking out two members of the Id family coexpressed in the development of embryonic vasculature (Id1 and Id3) caused embryonic lethality (27). Id3-deficient mice with one functional copy of Id1 did not die in development, but they were not able to support tumor growth and metastasis. Transplantation of bone marrow of the wild-type mice restores their ability to support tumor angiogenesis, growth, and metastasis (28).

### Trafficking of EPCs in Cancer

Tumor-derived paracrine signals activate the bone marrow compartment, resulting in the mobilization and recruitment of EPCs to the tumor bed. EPCs have to accomplish three distinct but interrelated steps during vasculogenesis: mobilization from the bone marrow to the peripheral blood; homing and invasion of tumor site; and differentiation into mature endothelial cells and/or regulation of preexisting endothelial cells via paracrine or juxtacrine signals (22). During these steps, EPCs interact with different physiologic compartments, including bone marrow, peripheral blood, blood vessels, and cancer tissues. The success of each step depends on the ability of EPCs to interact, adapt, and respond to other cells in these compartments (29).

#### Mobilization of EPCs from bone marrow to peripheral blood

In normal conditions, EPCs reside within a stem cell niche in the bone marrow, where fibroblasts, osteoblasts, and endothelial cells regulate the maintenance and mobilization of the bone marrow stem cells (30). The release of EPCs from the bone marrow is regulated by a variety of growth factors, enzymes, ligands, and surface receptors. Figure 3 summarizes the cytokines, proteases, and integrins involved in the mobilization of EPCs from bone marrow to peripheral blood.

**Cytokines.** Cytokines inducing mobilization interfere with the interactions between EPCs and bone marrow stromal cells (BMSC), which allow EPCs to disengage the bone marrow and to pass through the sinusoidal endothelium to enter the bloodstream (31). EPCs mobilize into the peripheral blood in response to tumor cytokines and move to the tumor bed, where they incorporate into sprouting neovessels (23).

Tissue injury and hypoxia cause the production and release of factors responsible for mobilization of EPCs from the bone marrow (32). EPC mobilizing factors are released from tumors in a high concentration greater than that in the bone marrow, and the most described are VEGF, granulocyte colony-stimulating factor (G-CSF), basic fibroblast growth factor (bFGF), placental growth factor (PIGF), Epo, and SDF-1 (22, 31, 33). Drugs, such as statins, can induce mobilization of EPCs (34) in a mechanism that requires eNOS (ref. 35). Estrogen did not increase EPC mobilization...
in eNOS-deficient mice (33), although estrogen increased the number of circulating EPCs in wild-type mice. These data suggest that eNOS plays a fundamental role in the regulation of EPC mobilization from the bone marrow (36).

One of the most important regulators of the mobilization of EPCs is VEGF. Its expression is markedly increased in hypoxic tissues and tumors largely because of the effects of hypoxia-inducible factor-1 (HIF-1) on VEGF transcription (37). VEGF binds to its receptor VEGFR-2 and mediates the further maturation of the hemangioblast/anglioblast cascade and early/late EPCs (15). VEGF also activates matrix metalloproteinase-9 (MMP-9) which confirms its key role in the process of cell invasion (38). In the stem cell niche, cells are exposed to high levels of SDF-1 (a chemoattractant for EPCs that binds via the CXCR4 that maintains the cells in the niche; ref. 39).

**Proteases.** EPC mobilization is mediated by proteases such as elastase, cathepsin G, and matrix metalloproteinase (MMP; ref. 40). G-CSF releases these proteases from neutrophils, and these proteases cleave the cytokine SDF-1 (41). The proteases elastase and cathepsin G induce cleavage of adhesive bonds on stromal cells, which interact with integrins on hematopoietic stem cells (42). The EPC mobilization activity of VEGF and SDF-1 was shown to be MMP-9–dependent (38). Stromal cells are stimulated by EPC-mobilizing factors, which activate phosphoinositide 3-kinase/protein kinase (PI3K/AKT) pathway and eNOS, leading to an increased production of nitric oxide that stimulates and maintains MMP-9 activity (36, 38). In particular, the initial step in the mobilization of EPCs from the bone marrow begins with the activation of MMP-9, which cleaves the membrane-bound Kit ligand (mKit-Lig) to a soluble form (sKit-Lig), which binds to c-Kit receptor on EPCs and activates the detachment between EPCs and BMSCs and the intravasation of EPCs from the vascular zone of the bone marrow to the peripheral blood. The proteases elastase and cathepsin G induces cleavage of adhesive bonds on BMSCs, which interact with integrins in EPC retention such as α4β1 and β3. G-CSF releases proteases from neutrophils that cleave the cytokine SDF-1 in the bone marrow, which results in decreased adhesion of EPCs.

**Integrins.** Integrins also regulate different steps of EPC mobilization from the bone marrow (29): the integrin αβ3 mediates cell adhesion to vascular cell adhesion molecule-1 (VCAM-1) and cellular fibronectin and is a key regulator of EPC retention and mobilization from the bone marrow (44). Downregulation or functional blockade of αβ3 integrin–mediated EPC lodgment in the bone marrow causes mobilization of EPCs (45). Similarly, VEGF-induced mobilization of EPCs involves downregulation of β3-integrin in bone marrow (46).

**Homing of EPCs to tumor bed**

Once in the peripheral blood, EPCs home to tumor tissues in response to chemokine gradients that are formed in ischemic or hypoxic tumor tissues (29) where they can participate in neovascularization (12). Figure 4 summarizes...
the cytokines, receptors, adhesion molecules (integrins and selectins), and proteases involved in the homing of EPCs to the tumor bed.

Chemotaxis. The major chemokines and their respective receptors that regulate EPC activation and homing are VEGF/VEGFR-2 (47), SDF-1/CXCR4 (48), interleukin (IL)-8/CXCR2 (49), GRO-α/CXCR1 (50), CCR2/CCL2, and CCR5/CCL5 (51). VEGF was shown to upregulate SDF-1 and CXCR4 (52); with low VEGF, SDF-1 was insufficient for homing of EPCs to tumor sites (53), showing possible synergistic effect between the two cytokines.

Under hypoxic conditions, transcription factors such as HIF-1 were activated and led to increased transcription of VEGF and SDF-1α (47). HIF-1α, SDF-1, and VEGF were shown to be important regulators of EPCs homing to hepatocellular carcinoma (54). By binding to the receptors CXCR1 and CXCR2, IL-8 enhanced endothelial cell survival and proliferation, increased MMP production, and increased capillary tube formation (55). CXCR2 and CCR5 are chemokine receptors expressed on the surface of EPCs and bind the ligands CCL2 and CCL5, respectively. These chemokines and their receptors are involved in EPC migration and differentiation and play an important role in vascular remodeling and angiogenesis (56).

Extravasation. EPC homing is an active process involving direct interaction between molecular targets expressed by EPCs and by tissues that they home to (57). After chemotaxis, EPCs become activated and start selectin-initiated and integrin-mediated adhesion to endothelial vascular cells resulting in transendothelial migration into sites where vascular remodeling is needed (29). P-selectin glycoprotein ligand-1 (PSGL-1) expressed on the surface of EPCs interacts with P-selectin and E-selectin expressed by tissues that they home to via an RGD motif-dependent, CCL5, and

Figure 4. Homing of EPCs to tumor bed. EPCs migrate to tumor in a chemotaxis response to tumor-secreted cytokines (VEGF, SDF-1, IL-8, GRO-α, CCL5, and CCL2) which interact with their respective receptors (VEGFR-2, CXCR4, CXCR2, CXCR1, CCR5, and CCR2) on EPC surface. After chemotaxis, PSGL-1 interacts with P-selectin and E-selectin expressed on endothelial cells and facilitates the initial interaction of EPCs with the blood vessel wall; this activates integrins to mediate intercellular adhesion to facilitate EPC transendothelial migration. \( \alpha_{5}\beta_{1}\)- and \( \alpha_{6}\beta_{1}\)-integrins mediate the adhesion of EPCs to endothelial molecules such as ICAM-1, FG, fibronectin, and laminin. Also, \( \alpha_{v}\beta_{3}\)- and \( \alpha_{v}\beta_{5}\)-integrins expressed on mesenchymal cells play a role in EPC homing, due to the binding to RGD motif regions of different ligands. Finally, integrins and proteases are essential to facilitate the EPC tissue invasion. Uregulation of integrins \( \alpha_{5}\beta_{1}\) and \( \alpha_{6}\beta_{1}\) mediates EPC invasion and migration toward VEGF gradients within the ECM in a PI3K/AKT pathway-dependent way and also extracellular proteases are involved in EPC invasion such as MMP-9, cathepsin L, or urokinase- and tissue-type plasminogen activators.
a fibronectin receptor highly expressed on EPCs and participates in homing to vascular injury sites and promotes re-endothelialization (60). αβ1-integrin is a laminin-binding integrin regulated by VEGF and bFGF and necessary for EPC homing and adhesion to the basement membrane (61). Integrin αv is expressed on almost all the cells originating from the mesenchyme and plays a significant role in EPC homing. More specifically, αvβ3- and αvβ5-integrins bind to arginine–glycine–aspartic acid (RGD) motif regions of different ligands including vitronectin, fibronectin, osteopontin, FG and vWF. (58). Once adhered at specific homing sites, EPCs need to migrate through the endothelial monolayer, a step mediated by β2-integrins and dependent upon VEGF and increased expression of ICAM-1 in endothelial cells (62).

**Tissue invasion.** EPCs need to migrate through the blood vessel basement membrane and through the extracellular matrix (ECM) to home to sites where they need to exert their functions (29). These processes require interactions with cells and ECM, wherein integrins and extracellular proteases are essential to modulate EPC invasion (63). The major extracellular proteases involved in EPC invasion are members of the MMP family (MMP-9; ref. 64), the cathepsin family (cathepsin L; ref. 65), and the serine protease family (urokinase- and tissue-type plasminogen activators; ref. 66). EPCs upregulate the production of extracellular proteases that allow both matrix degradation and EPC migration (63). In addition, upregulation of integrins α5β1 and α6β1 mediates EPC invasion and migration toward VEGF gradients within the ECM by the PI3K/AKT pathway (67).

**In situ differentiation and paracrine/juxtacrine factor production**

EPC differentiation can be divided in three sequential stages: integrin-mediated adhesion to specific ECM components, growth factor–induced proliferation and survival, and maturation and functional acquisition of endothelial cell properties (68). Figure 5 summarizes the cytokines and integrins implicated in the differentiation into mature endothelial cells and that regulate preexisting endothelial cells via paracrine or juxtacrine signals.

EPC adhesion to the ECM is an essential step during differentiation, and direct interaction between integrins and ECM can regulate EPC paracrine/juxtacrine factor production (29). All the integrins implicated are fibronectin-binding integrins; therefore, the interaction of EPCs with fibronectin is essential during endothelial differentiation (11, 29). Fibronectin is described as a major regulator of EPC differentiation, as it promotes VEGF-induced differentiation of EPCs into endothelial cells via specific binding to integrin α5β1 (69). Fibronectin downregulates the expression of integrins α5β1 and αvβ5 during EPC differentiation (70). Paracrine/juxtacrine factor production by EPCs is also regulated by the integrin–ECM interactions (71). Activation of integrin β3 in EPCs induced expression of proangiogenic factors such as IL-8 and CCL2 (67, 72). EPCs contribute to new vessel formation and remodeling by differentiation into mature endothelial cells and regulation of preexisting endothelial cells and other cell types with production of paracrine and/or juxtacrine signals, such as VEGF, SDF-1, platelet derived growth factor-1 (PDGF-1), CCL2, and insulin-like growth factor 1 (IGF-I; ref. 73).
The maturation and acquisition of an endothelial phenotype depends mainly on the regulation of the transcription factor HoxA (74). HoxA regulates the expression of the endothelial genes for eNOS, VEGFR-2, and VE-cadherin by transcriptional regulation of histone deacetylases (HDAC; ref. 74). These steps lead to an overall response that promotes differentiation and are highly regulated by processes involved in tumor angiogenesis.

Summary

EPCs are essential for tumor progression and metastasis; they promote both angiogenic signals and structural support for existing endothelial cells to promote vascularization. EPCs migrate from the bone marrow to the tumor site; during the process, they express different markers and highly regulate processes involved in chemotaxis, adhesion, and invasion. Hypoxic tumors secrete cytokines that activate the bone marrow (EPCs and BMSCs) to promote de-adhesion and intravasation of EPCs to the circulation through downregulation of adhesion signals and activation of proteases. While in the circulation, EPCs respond to tumor-secreted cytokines for homing by activation of chemotaxis through chemokine receptors, extravasation through selectins and integrins, and invasion through integrins and proteases. After homing, EPCs differentiate to mature endothelial cells and promote vascularization. This review summarizes the expression and the function of the various surface markers, cytokines, receptors, adhesion molecules, proteases, and cell signaling mechanisms involved in the different steps (mobilization, homing, and differentiation) of EPC trafficking from the bone marrow to the tumor site. Understanding the biologic mechanisms of EPC cell trafficking opens a window for new therapeutic targets in cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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