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Angiogenesis Review Series

The involvement of endothelial progenitor cells in tumor angiogenesis

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Abstract

Endothelial progenitor cells (EPCs) have been isolated from peripheral blood CD34, VEGFR-2, or AC 133 (CD133) antigen-positive cells, which may home to site of neovascularization and differentiate into endothelial cells *in situ*. Endothelial cells contribute to tumor angiogenesis, and can originate from sprouting or co-option of neighbouring pre-existing vessels. Emerging evidence indicate that bone marrow-derived circulating EPCs can contribute to tumor angiogenesis and growth of certain tumors. This review article will summarize the literature data concerning this new role played by EPCs in tumor angiogenesis.

Keywords: angiogenesis - endothelial progenitor cells - tumor

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Early development of the endothelial and hematopoietic lineage are closely linked

There is a close relationship between the development of blood and endothelium, indicating that hematopoietic cells (HCs) and endothelial cells (ECs) come from a common progenitor, the hemangioblast, a transient cell stage that develops early and disappears quickly during embryonic development [1]. The ontogenic development of hemangioblast is linked to vascular endothelial growth factor receptor-2 (VEGFR-2) because the mice lacking VEGFR-2 display a defect in both hematopoietic cells and vasculature [2,3]. VEGFR-2 positive cells, isolated from the chick embryo mesoderm at the gastrulation stage, give rise to either HC or EC colonies [4]. Moreover, the VEGFR-2 positive cells isolated from differentiating embryonic stem cells generate mixed hematopoietic-endothelial colonies in unicellular culture [5].

Further studies have indicated that in human postnatal life AC133 (CD 133), CD34 and VEGFR-2 positive cell subsets in bone marrow, peripheral blood, and cord blood also possess the functional activity of hemangioblast, since they are able to differentiate into both HCs and ECs [6].

The discovery of endothelial progenitor cells

Extensive data support the existence of endothelial progenitor cells (EPCs), their bone marrow origin, and contribution to the formation of new blood vessels in adults. Moreover, their discovery led to the new concept that vasculogenesis and angiogenesis may occur simultaneously in the postnatal life because these cells are able to differentiate when needed into vascular endothelium, through a mechanism recapitulating embryonic vasculogenesis [7,8].

The majority of circulating EPCs reside in the bone marrow in close association with hematopoietic stem cells and the bone marrow stroma that provides an optimal microenvironment. EPCs have the capacity to proliferate, migrate and differentiate into endothelial lineage cells, but have not yet acquired characteristics of mature EC.

Differences between EPCs and circulating ECs

Circulating ECs were first demonstrated in the 1960 using Dacron grafts placed in the pig, rabbit and dog [9]. ECs lining the coronary arteries of a transplanted human heart were then shown to be derived from the recipient and not the donor [10]. ECs have since been shown to line a ventricular assist device [11]. In this context it is interesting that circulating ECs are detectable in diseases marked by vascular injury, namely sickle cell anemia, acute myocardial infarction, thrombotic thrombocytopenic purpura, and active cytomegalovirus infection [12–15]. Normal adults have 2.6±1.6 circulating ECs per mm of peripheral blood [15]. Most of these cells are quiescent, and at least half are microvascular as defined by CD36 positivity [15].

Circulating EPCs differ from the circulating ECs that are randomly detached from the vessel walls and enter the circulation as a result of vascular injury. Moreover, when EPCs were exposed to angiogenic factors, they formed highly proliferative endothelial colonies, whereas circulating EC could only generate endothelial monolayers that had limited proliferation capacity because they are mature, terminally differentiated cells [15–17].

Markers of EPCs

Studies in quail/chick chimeras showed that fibroblast growth factor-2 (FGF-2) mediates the induction of EPCs from mesoderm [18]. ECPs were initially identified and isolated on the basis of their expression of VEGFR-2 and CD34 shared by the angioblast and the hematopoietic progenitors [7]. These ECPs were subsequently shown to express VE-cadherin and AC 133 (CD 133), an orphan receptor that is specifically expressed on EPCs, but whose expression is lost once they differentiate into more mature ECs [19]. CD133 and VEGFR-2 positive EPCs were found to be present at low frequencies in human umbilical cord blood [19], adult bone marrow [19–21], human fetal liver cells [19] and cytokine-mobilized peripheral blood [19, 20].

When incubated *in vitro* with appropiate medium in the presence of specific growth factors, circulating EPCs have a high proliferating potential and within 10 to 20 days produce colonies of cells expressing endothelial cell markers, such as CD34 [8, 22], AC 133 (CD 133) [20] and VEGFR-2 [19]. When incubated with VEGF, FGF-2 and insulin-like growth factor (IGF) on collagen/fibronectin coated dishes, CD133 positive cells downregualate CD133 expression and differentiate into mature adherent ECs, which express endothelial specific markers, such as von Willebrand factor (vWF) and VE-cadherin.

Peichev *et al.* [19] demonstrated that a small subset of CD34 positive cells from different hematopoietic sources express both CD133 and VEGFR-2. Incubation of this subset with VEGF, FGF-2 and collagen results in their proliferation and differentiation into CD133 negative VEGFR-2 positive mature ECs. Maturation and *in vitro* differentiation of these cells abolish CD133 expression, suggesting that EPCs with angioblast potential may be marked selectively with CD133.

Gehling *et al.* [20] showed that CD133 positive cells from granulocyte-macrophage colony-stimulating factor (GM-CSF)-mobilized peripheral blood differentiate into ECs when cultured in the presence of VEGF and stem cell growth factor. Phenotypic analysis revealed that most of these cells display endothelial features, including the expression of VEGFR-2, Tie-2 and vWF. All these data indicate that CD133 is currently the best selective marker for identifying ECPs and that circulating CD34, VEGFR-2 and CD 133 positive cells constitute a phenotypically and functionally distinct population of circulating ECs that may play a role in postnatal vasculogenesis.

Recruitment of EPCs to sites of active neovascularization

EPCs mobilized from the bone marrow into the blood stream may be recruited and incorporated into sites of active neovascularization during tissue ischemia, vascular trauma or tumor growth [8, 19, 23–31]. Reports on the numeric contribution of EPCs to vessel growth are variable, ranging from very low (<0.1%) to high (up to 50%) likely dependent on the type of angiogenesis model used [32,33].

Triggers of EPCs recruitment

EPCs recruitment to sites of neoangiogenesis is triggered by the increased availability of angiogenic growth factors or chemokines, such as VEGF, angiopoietin and stromal cell-derived factor (SDF)-1α [34–36]. The latter binds to the chemokine receptor CXCR-4, which is highly expressed on EPCs [37]. Once avoided at the site of neovascularization, EPCs may recruit additional EPCs by releasing growth factors, such as VEGF, hepatocyte growth factor (HGF), granulocyte-colony stimulating factor (G-CSF) and GM-CSF [38]. Moreover, angiogenic factors activate matrix metalloproteinases (MMPs), specifically MMP-9 [39], which lead to the release of soluble KIT ligand (sKITL) [40] which, in turn, promotes the proliferation and motility within the bone marrow microenvironment, thereby laying the framework for their mobilization to the peripheral circulation.

Mechanisms of tumor angiogenesis

Tumor progression and maintenance requires the development of an ample blood supply, which ensures the delivery of oxygen, nutrients and growth factors. Tumor angiogenesis is linked to a switch in the equilibrium between positive and negative regulators. In normal tissues, vascular quiescence is maintained by the dominant influence of endogenous angiogenesis inhibitors over angiogenic stimuli. Tumor angiogenesis, on the other hand, is induced by increased secretion of angiogenic factors and/or downregulation of angiogenesis inhibitors [41].

Tumour growth consists of an avascular and a subsequent vascular phase. Assuming that it is dependent on angiogenesis and that this depends on the release of angiogenic factors, acquisition of angiogenic capability can be seen as an expression of progression from neoplastic transformation to tumor growth and metastasis [41].

Contribution of EPCs to tumor angiogenesis

The current wisdom is that tumors are endowed with angiogenic capability and that their growth, invasion and metastasis are angiogenesis-dependent. Recent morphological studies indicate that some tumors may be vascularized without significant angiogenesis, probably by using existing vessels, a process later described as vascular co-option, or even by forming vascular channels on their own through a non-endothelial cell process called "vascular mimicry" [42]. The notion of "vascular mimicry" has been proposed to account for the ability of tumor cells to serve a vascular function within the tumor.

In addition to the sprouting and co-option of neighbouring pre-existing vessels, tumor angiogenesis is supported by the mobilization and functional incorporation of EPCs. The recruitment of EPCs to tumor angiogenesis represents a multistep process, including: i) active arrest and homing of the circulating cells within the angiogenic microvasculature; ii) transendothelial extravasation into the interstitial space; iii) extravascular formation of cellular clusters; iv) creation of vascular sprouts and cellular networks; v) incorporation into a functional microvasculature.

The fact that EPCs can contribute to tumor angiogenesis indicates that EPCs, although they are primarily programmed to form blood vessels during embryonic vascular development, retain this ability within an angiogenic environment in the adult. EPCs have been detected at increased frequency in the circulation of cancer patients and lymphoma-bearing mice and tumor volume and tumor production of VEGF were found to be correlated with EPCs mobilization [43, 44].

Vajkoczy *et al.* [45] investigated the mechanisms of homing and incorporation of EPCs during new blood vessel formation in a tumor model using mouse embryonic EPCs as a model system and they showed that EPCs retain their ability to contribute to tumor angiogenesis in the adult. Circulating EPCs are specifically arrested in 'hot spots' within the tumor microvasculature, extravasate in the interstitium, form multicellular clusters, and incorporate into functional vascular networks.

Bone marrow derived cells, including EPCs, restored impaired VEGF-driven angiogenesis in mice lacking placental growth factor (PlGF) [46]. PlGF null mice have impaired tumor angiogenesis due to decreased vessel density. When these mice received bone marrow transplantation from wild-type mice, they were able to support tumor angiogenesis, indicating that PlGF is delivered to tumor vasculature by bone marrow-derived cells.

The percentage of EPCs incorporation is generally low and depends on the nature of the tumor, supporting the concept that most tumor neovascularization sems to occur via angiogenesis. However, in some model systems, tumors are reliant on EPCs mobilization [29].

High levels of VEGF produced by tumors may result in the mobilization of bone marrow-derived stem cells in the peripheral circulation and enhance their recruitment into the tumor vasculature [23, 34].

Hypoxia can also mobilize EPCs from the bone marrow in the same way, as hematopoietic cytokines, such as GM-CSF [31]. Malignant tumor growth results in neoplastic tissue hypoxia, and may mobilize bone marrow-derived ECs in a paracrine fashion and thus contribute to the sprouting of new tumor vessels.

When EPCs are engrafted into immunocompromised mice, they incorporate into the vasculature of xenotransplanted tumors. To distinguish between the contribution of bone marrow-derived EPCs and that of neighbouring vessels many studies have taken advantage of transplantation techniques in which sex-mismatched or genetically marked donor bone marrow is infused into irradiated recipients. Id 1/3 double-mutant mouse embryos have vascular malformations in the forebrain, leading to fatal haemorrhage. Adult mice with reduced Id gene dosage cannot support tumor-induced angiogenesis [29]. Lyden *et al.* [29] demonstrated that transplantation and engrafment of β-galactosidase-positive wild-type bone marrow or VEGF-mobilized stem cells into lethally irradiated Id-mutant mice is sufficient to reconstitute tumor angiogenesis. In contrast to wild-type mice, Id-mutants fail to support the growth of tumors because of impaired angiogenesis. Tumor analysis demonstrates uptake of bone marrow-derived VEGFR-2-positive EPCs into vessels surrounded by VEGFR-1-positive myeloid cells. Defective angiogenesis in Id-mutant mice is associated with impaired VEGF-induced mobilization and prolif-

eration of the bone marrow precursor cells. Inhibition of both VEGFR-1 and VEGFR-2 signalling is needed to block tumor angiogenesis and induce necrosis. The mechanism for underlying angiogenesis defect is believed to be due, in part, to impaired recruitment of EPCs to the tumor. Both B6RV2 lymphoma and Lewis lung carcinoma cells, which fail to grow tumors in Id-deficient mice, are able to form fully vascularized tumors in Id-mutant mice that have received wild-type bone marrow transplants.

In another experimental approach human multipotential progenitor cells (MAPCs), which are highly primitive cells that have the capacity to differentiate into different cell types, were induced to differentiate into ECs. Reyes *et al.* [30] found that *in vitro*-generated MAPCs respond to angiogenic stimuli by migrating to tumor sites and contributing to tumor vascularization. MAPCs were injected into immunocompromised mice that carried mouse Lewis lung carcinoma. After 5 days, 30% of the newly-formed tumor-associated vessels were derived from human MAPC-derived ECs. Reyes *et al.* [30] also found that *in vivo* angiogenic stimuli in a tumor microenvironment are sufficient to recruit MAPCs to the tumor bed and induce their differentiation into ECs that contribute to the tumor vasculature. In fact, MAPCs contributed to the neovasculature of spontaneously formed lymphomas that commonly develop in ageing immunocompromised mice.

Asahara *et al.* [8] engrafted tumor-bearing mice with transgenic bone marrow cells in which constitutive LacZ expression is under the transcriptional regulation of VEGFR-2 and Tie-2 endothelial specific promoters. Histological examination of the tissues in growing tumors after bone marrow transplantation has shown localization of VEGFR-2 and Tie-2 expressing endothelial lineage cells derived from bone marrow in blood vessels and stroma around vasculature, indicating that bone marrow-derived cells, such as EPCs, can home to the tumor vasculature and differentiate into ECs.

Therapeutic implications

Inhibition of VEGFR-2 signaling results in impaired mobilization and recruitment of EPCs to tumor vasculature and is highly effective in retarding the growth of certain tumors.

As EPCs are endowed with the capacity to home the tumor vasculature, they might be used to deliver toxins. Under study-state conditions, the number od EPCs in the bone marrow or circulation is very low. Injection of chemotactic factors, such as VEGF or PlGF might be useful to mobilize larger numbers of EPCs for *in vitro* manipulation and therapeutic targeting of the tumor vasculature.

Future directions and perspectives

Little is known about which tumor types are most dependent on EPCs for their growth. Mobilization and functional incorporation of EPCs into the tumor vasculature is essential for the growth of lymphomas, Lewis lung carcinoma and colon cancers. Additional studies are required to identify the tumor types that are partially or fully dependent on the recruitment of EPCs.

Identification of chemokines/cytokines and tissue-specific extracellular matrix components that are involved in the recruitment of EPCs will provide new targets for the treatment of tumors.

It remains to be determined whether the number of EPCs and the molecular determinants of EPCs mobilization have any prognostic and therapeutic value for the treatment of certain malignancies.

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