Biochimica et Biophysica Acta 1846 (2014) 26–39

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Role of endothelial progenitor cells in cancer progression

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ARTICLE INFO

Article history:
Received 29 October 2013
Received in revised form 19 February 2014
Accepted 27 March 2014
Available online 4 April 2014

Keywords:
Endothelial progenitor cells
Cancers
Hematological malignancies
Vasculogenesis

ABSTRACT

Tumor-associated neovasculature is a critical therapeutic target; however, despite significant progress made in the clinical efficacy of anti-vessel drugs, the effect of these agents remains transient: over time, most patients develop resistance, which inevitably leads to tumor progression. To develop more effective treatments, it is imperative that we better understand the mechanisms involved in tumor vessel formation, how they participate to the tumor progression and metastasis, and the best way to target them.

Several mechanisms contribute to the formation of tumor-associated vasculature: i) neoangiogenesis; ii) vascular co-option; iii) mosaicism; iv) vasculogenic mimicry, and v) postnatal vasculogenesis. These mechanisms can also play a role in the development of resistance to anti-angiogenic drugs, and could serve as targets for designing new anti-vascular molecules to treat solid as well as hematological malignancies. Bone marrow-derived endothelial progenitor cell (EPC)-mediated vasculogenesis represents an important new target, especially at the early stage of tumor growth (when EPCs are critical for promoting the "angiogenic switch"), and during metastasis, when EPCs promote the transition from micro- to macro-metastases. In hematologic malignancies, the EPC population could be related to the neoplastic clone, and both may share a common ontogeny. Thus, characterization of tumor-associated EPCs in blood cancers may provide clues for more specific anti-vascular therapy that has both direct and indirect anti-tumor effects. Here, we review the role of vasculogenesis, mediated by bone marrow-derived EPCs, in the progression of cancer, with a particular focus on the role of these cells in promoting progression of hematological malignancies.

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http://dx.doi.org/10.1016/j.bbcan.2014.03.005
0304-419X/

1. Introduction

Vasculogenesis was first described as a phenomenon occurring in early embryogenesis, and was believed to not occur in adult tissues [1–3]. In 1997, Asahara et al. [4] purified a population of circulating cells that displayed properties of endothelial cells (ECs) as well as progenitor cells, and identified these cells as 'endothelial progenitor cells' (EPCs); these cells can differentiate into ECs *via* the process of postnatal vasculogenesis, as described *in vitro*, as well as *in vivo*, in a model of hind limb ischemia [4]. Ashara's report comprised the first demonstration that vasculogenesis contributes to vascular remodeling, as well as to *de novo* formation of vessels in postnatal life. In 1998, Shi et al. [5] suggested that vasculogenesis may have physiological and pathological roles in healthy and disease states in adults. Here we examine the role of EPCs and vasculogenesis in the progression of cancer, with particular attention to how they promote the progression of hematological malignancies.

1.1. Characteristics of circulating EPCs and their contribution to angiogenesis

Circulating EPCs reside in the bone marrow (BM), in close association with hematopoietic stem cells (HSCs) and the surrounding BM stromal milieu [6,7]. EPCs are derived from hemangioblasts, which are precursor cells that give rise to both EPCs and HSCs [8]. EPCs have the capacity to proliferate, migrate and differentiate into ECs [9]. Mobilization of EPCs may occur in response to low oxygenation in tissues (for example in response to tissue ischemia after myocardial infarction) or in response to tumor growth, leading to increased angiogenesis [9]. Mobilization of EPCs is followed by vasculogenesis, which occurs when circulating EPCs are recruited in response to factors secreted by ischemic tissues and by inflammatory and tumor cells, resulting in the generation of new vessels in injured or pathological tissues [7]. It has been hypothesized that once EPCs are recruited to tumor sites, they can sustain neovessel formation *via* paracrine secretion of pro-angiogenic growth factors [10]; also that these cells provide structural support by being directly incorporated into the lumens of sprouting nascent vessels [11,12]. However, the extent to which EPCs contribute to blood vessel formation in postnatal life (vasculogenesis) is controversial. The number of BM-derived EPCs incorporated into tumor neovessels reportedly ranges from significant to undetectable, even when the same tumor models are used by different investigators [13]. For instance, EPC contributions as high as 50% [14,15] to as low as 5%–20% [16–19], and in some cases even undetectable [20–24], have been reported. These differences are likely based on a number of technical and experimental variables. The use of non-standardized methodologies (flow cytometry or *in vitro* methods) for the quantification of circulating EPCs add to the uncertainty [25]. We believe that many of these problems could be rectified if we had markers to specifically identify the putative EPC population (including circulating and resident EPCs). None of the markers currently used for such identification are restricted to EPCs: all antigens or combinations of antigens used for this purpose are also expressed by circulating HSCs and progenitor cells, circulating mature ECs, platelets/EC-derived vesicles, and some subsets of circulating hematopoietic-derived monocyte/myeloid cells (Table 1) [26,27]. Thus, in the absence of unique antigens to identify the EPCs, it is possible that cells that were previously identified as circulating EPCs actually represent circulating hematopoietic-derived cells, which express both hematopoietic and endothelial cell markers [25,27]: these include "vasculogenic monocytes", or Tie2-expressing monocytes, neutrophils, dendritic cell (DC) precursors, and Gr1+CD11b+ "myeloid-derived suppressor cells [MDSCs]"). All of these hematopoietic-derived cell subsets reportedly also have pro-vasculogenic activities, further supporting the possibility that prior studies that labeled cells as EPCs may have included several BM-derived cell populations of hematopoietic origin, thereby explaining most of the controversies in the field [28]. Note that such miscategorization is not surprising, because EPCs and hematopoietic progenitor cells are derived from a common precursor, the hemangioblast — thus at early stages of differentiation, EPCs and hematopoietic-derived cells may share phenotypic as well as functional characteristics, and may both have the capacity to contribute to neovessel formation in the presence of proper stimuli.

Circulating EPCs differ from circulating mature ECs (CECs). CECs are randomly detached from vessel walls, and enter the circulation subsequent to vascular injury [29]. Also, when EPCs are exposed to angiogenic factors, they give rise to highly proliferative endothelial colonies, whereas similarly exposed CECs can only generate endothelial monolayers, which have a limited proliferative capacity due to their terminally differentiated phenotype [30,31]. Finally, when circulating EPCs are recruited to inflammatory or tumor sites, they differentiate into mature ECs and integrate into nascent neovessels, while CECs lack this ability.

Table 1
Principal surface markers used to characterize circulating endothelial progenitor cells (EPCs) and comparison of their expression in adult hematopoietic stem cells and mature endothelial cells.

Markers	Name	Adult hematopoietic stem cells	Circulating endothelial progenitor cells	Endothelial cells
CD34	-Hematopoietic Progenitor Cell Antigen CD34	Positive	Positive	Positive
VEGFR2 (KDR)	-Fetal liver kinase 1 (FLK1) -CD309	Positive (a subpopulation)	Positive	Positive
CD45	-Leukocyte common antigen (LCA)	Positive	Positive, or dim positive, or negative	Negative
CD133	-Prominin-1 -AC133	Positive	Positive, or negative	Negative
CXCR4	C–X–C chemokine receptor type 4 (CXCR-4) -fusin -CD184	Positive	Positive	Positive
VE-Cadherin	-Cadherin 5, type 2 -CD144	Negative	Positive, or negative	Positive
CD31	-Platelet endothelial cell adhesion molecule (PECAM-1)	Positive	Positive	Positive
CD146	-Melanoma cell adhesion molecule (MEL-CAM) -MUC-18	NR	Positive, or negative	Positive
Tie-2	-Tunica Interna Endothelial Cell Kinase -TEK Tyrosine Kinase -VMCM1	Positive	Positive	Positive
CD14	-Myeloid cell-specific leucine-rich glycoprotein	Negative	Negative	Negative
CD105	-Endoglin (END) -FLJ41744 -HHT1 -ORW1	Positive	Positive	Positive

NR: not reported.

1.2. Methods for EPC isolation in vitro

Several *in vitro* methods have been developed to select and expand the putative population of EPCs [30,31]; through these methods at least three different cell populations can be isolated from total peripheral blood mononuclear cells (PBMCs), all of which are related to vasculogenesis: the colony-forming unit-ECs (CFU-ECs) or colony-forming unit-Hill (CFU-Hill) cells; the circulating angiogenic cells (CACs); and the endothelial colony-forming cells (ECFCs) (see Fig. 1 and Table 2 for a detailed explanation of EPC *in vitro* isolation and cell culture methods) [30,31]. CACs and CFU-ECs consistently display a mixed endothelial – monocytic/hematopoietic phenotype [32–36], and their gene expression profiles are similar to those of cultured monocytes that have been exposed to pro-angiogenic factors, but differ from those of cultured ECs [37,38].

In contrast, ECFCs are phenotypically indistinguishable from cultured ECs, and possess *de novo* vessel-forming ability (Table 2), as shown in an *in vivo* collagen gel-based assay [33,34]; this ability has not yet been attributed to CFU-ECs [33]. These findings led to the conclusion that ECFCs – more recently renamed endothelial outgrowth cells (EOCs) – display features that are consistent with the EPC-related phenotype [33,34] (Fig. 1 and Table 2); on the other hand, CACs and EC-CFUs represent mixed subsets of peripheral blood (PB) hematopoietic-derived cells (monocytes/macrophages, myeloid and lymphoid progenitor cells), which can indirectly promote vessel growth/repair [27]. This may explain why, although EC-CFUs may not indirectly quantify putative EPCs, still the frequency of their colony formation in human PB is highly correlated to adverse cardiovascular risk in human subjects with cardiovascular disease [39].

Finally, a disadvantage of the study of *in vitro* cultured "EPCs" is that these cells may have acquired or lost properties during the culture process, as compared to their non-cultured counterparts (namely the circulating EPCs, from which they originate). This consideration in part explains some controversies in the field of EPC transplantation, and elucidates why many studies have failed to demonstrate the therapeutic potential of *in vitro* cultured EPCs in patients with ischemic cardiac diseases [40].

1.3. Use of flow cytometry to quantify circulating EPCs

Flow cytometry is widely used to quantify and study circulating EPCs [36]. However, the lack of EPC-specific markers makes it necessary to use a combination of markers, and unfortunately, the best combination is still far from being universally accepted [30]. As stated above, most of

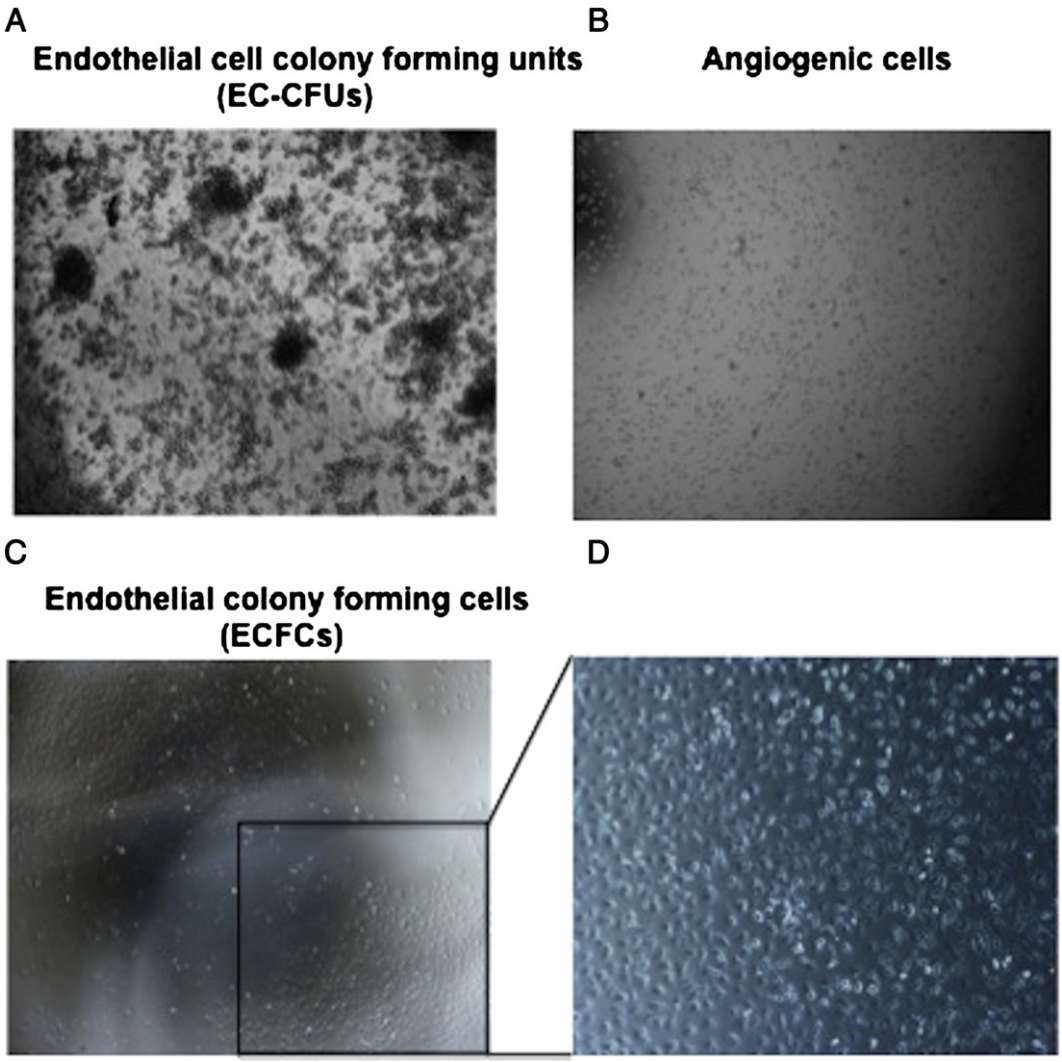

Fig. 1. Putative *in vitro* isolated endothelial progenitor cells. (A) Endothelial cell colony-forming units (EC-CFUs). Non-adherent mononuclear cells grown on fibronectin form colonies after 4 to 9 days (magnification 10×). (B) Angiogenic cells: whole, un-fractioned mononuclear cells are cultured on fibronectin in supplemented endothelial growth media for 4 days and then the non-adherent fraction of cells is removed (magnification 4×). (C and D) Endothelial colony-forming cells (ECFCs). Adherent mononuclear cells grown on collagen I display cobblestone morphology after 7 to 21 days; magnifications: 4× (C) and 20× (D).

Table 2
Characteristics of *in vitro* isolated subtypes of "endothelial progenitor cells (EPCs)".

Acronym	Name	Definition	Characteristics
Circulating EPCs	Circulating endothelial progenitor cells	Several cell types are included under this definition	-Circulating EPCs contribute to the formation of blood vessels, either by direct incorporation into vascular networks or indirectly, through paracrine activity on vessels. -Circulating EPCs include CD34 + CD133 + VEGFR2 + cells, subsets of monocytic cells, and cell populations with broad developmental plasticity.
EC-CFUs and CFU-Hill (early outgrowth cells)	Endothelial cell colony-forming units	Hematopoietic-derived monocytes and macrophages which display a mixed endothelial–monocytic/hematopoietic phenotype	-Colonies with a central core of "round" cells, with more elongated "sprouting" cells at the periphery, derived from PBMCs plated in fibronectin-coated dishes after a pre-plating step. -EC-CFU derived cells express EC specific markers together with the hematopoietic marker CD45 and the monocyte markers CD14 and CD115; they are not able to form vessels in *in vivo* collagen gel-based assay.
CACs (early outgrowth cells)	Circulating angiogenic cells	Hematopoietic-derived monocytes and macrophages which display a mixed endothelial-monocytic/ hematopoietic phenotype	-Cells derived from PBMCs plated in fibronectin-coated dishes and cultured in supplemented endothelial growth media for 4 days; after the non-adherent fraction of cells are removed, and CAC cells are obtained. -CACs do not form colonies but are functionally and phenotypically indistinguishable from cells derived from EC-CFUs.
ECFCs (late outgrowth cells)	Endothelial colony-forming cells	Endothelial cells with high proliferative activity	-Colonies with a cobblestone morphology derived from PBMCs plated on collagen I-coated dishes in medium containing endothelial growth factors without pre-plating steps (14–25 days cell culture). -ECFC-derived cells express EC antigens, including CD31, CD105, CD144, CD146, vWF, VEGFR2, and UEA-1. -ECFC-derived cells do not express CD45 and the monocyte markers CD14 and CD115 and are able to form vessels in *in vivo* collagen gel-based assay.

PBMCs: peripheral blood mononuclear cells, vWF: von Willebrand factor. UEA-1: Ulex Europaeus Agglutinin 1.

flow-markers used to identify EPCs are also shared by other circulating hematopoietic-derived cells (mostly myeloid cells), as well as by mature ECs (Table 1).

Accumulating evidence supports the concept that strictly interconnects hematopoietic and endothelial cells [41]: in particular, a hemogenic endothelium has been shown to generate hematopoietic cells, at least during development [42]. This discovery of the embryonic process of endothelial-to-hematopoietic transition (which may also occur during postnatal life) has important implications for the function and phenotype of EPCs [43]: it has been proposed that circulating putative EPCs, HSCs, and circulating hematopoietic-derived cells (all originating from the hemangioblast) may overlap phenotypically as well as functionally, and that different subsets of BM-derived cells may collaborate in vessel formation/repair under pathological conditions [26,27].

The lack of a consensus definition of circulating EPCs based on cell surface antigen expression has led to the proposal of different flow-cytometry protocols [25], in which an increasingly complex antigenic definition is used to characterize EPCs. It has been suggested that most of the flow-cytometry protocols previously used to identify putative EPCs actually quantified BM-derived cells of hematopoietic origin at various stages of differentiation [25], further highlighting current uncertainty in EPC definition. In theory, the use of complex flow-cytometry panels increases the specificity of EPC detection; however, it also leads to a lower reproducibility of the results [30], and in fact, the extreme rarity EPCs in the circulation greatly limits the use of complex flow-cytometry panels to quantify this cell type. Sample preparation (use of whole blood antibody staining, or ficoll separation or use red blood cell lysis buffer for isolation of PBMCs), gating controls (*i.e.* fluorescence minus one controls), optimization of the antibody panel, and compensation and the selection of logarithmic *versus* bi-exponential displays, may overcome this problem to some extent [44].

False positive events and readings of non-specific fluorescent events (circulating endothelial and platelet-derived extracellular vesicles, red blood cells, and auto-fluorescence along with non-specific antibody binding of dead cells) [44,45] represent additional possible confounds to be considered. Thus, despite improved flow-cytometry protocols have been recently proposed [44], the lack of specific EPC markers remains a major limitation in this field.

1.4. EPC markers

In humans, EPCs were initially identified and isolated on the basis of their expression of VEGFR-2 and CD34; however, expression of these markers is also shared by the hemangioblast and by hematopoietic progenitors [4]. EPCs were subsequently shown to also express other markers such as VE-cadherin, CXCR4, CD31, and AC133 (CD133), CD105, CD144, CD106, and CD117 (c-Kit) [7,46] (Table 1).

Studies of hemato-endothelial development show that CD34 + embryonic hemangioblasts do not express the common leukocyte antigen CD45; they acquire this marker only as they differentiate into hematopoietic progenitor cells (which become CD34 + CD45 +), but not if they become committed to the endothelial lineage [47–49]. Therefore, CD45 expression marks hematopoietic specification from fetal life into adulthood, but is not detected on cells of endothelial lineage [50]. Importantly, the CD34 + CD45 − cell fraction is able to generate ECFCs, while CD34 + CD45 + hematopoietic cells are not [51,52]. Recently, this was confirmed by a separate study, which showed that ECFCs are derived from CD34 + CD45 − mononuclear cell fraction (that co-expresses CD31, CD146 and CD105) or from the CD146 + CD45 − mononuclear cell fraction [44]. Thus, if ECFCs are derived from true EPCs, they should appear in the CD34 + CD45 − cell fraction, but not in the CD34 + CD45 + hematopoietic cell fraction. However, CD45 expression on EPCs remains to be confirmed [30]. Therefore, even the expression of CD45 − generally considered to be a specific pan-leukocyte marker, does not reliably differentiate between cells of the hematopoietic and endothelial progenitor lineage [41].

Peichev and co-workers [53] report that CD34 + VEGFR2 + cells that co-express CD133 represent true immature EPCs, and that as they mature, CD34 + VEGFR2 + CD133 + EPCs lose the expression of CD133; this is consistent with the evidence that mature ECs that line blood vessels in the adult, do not express CD133 [53]. In contrast, Timmermans and co-workers showed that the ECFCs generated by CD34 + CD45 − cell fraction do not express CD133 [52]; several other investigators have also been unable to detect the CD133 antigen on CD34 + CD45 − cells [54,55]. In fact, it was recently shown that CD34 + VEGFR2 + CD133 + cells are CD45 + hematopoietic progenitors, rather than true EPCs, and they do not generate ECFCs [51,52]; as a result, CD133 should no longer be considered as an EPC-specific marker. These contradictory reports make it difficult to interpret previous studies, in which EPCs were classically defined as CD133 + cells; this is especially true in the field of hematologic malignancies, where circulating EPCs have been consistently defined as circulating CD133 + cells.

Collectively, the above studies demonstrate that the identity of EPCs remains elusive and that the term "EPC" is currently a generic term, which needs to be interpreted based on the methodological approaches used to identify these cells.

2. EPCs in physiological conditions and in non-cancer diseases

In healthy individuals, the number of circulating EPCs is extremely low (less than 0.01% to 0.001% of PBMCs, by flow cytometry) [30]. Aging subjects and males have lower levels of CD34 + VEGFR2 + EPCs than found in young individuals [56] and in females [57], respectively.

Cardiovascular risk factors together cause a pro-inflammatory state and endothelial dysfunction, which lead to premature atherosclerosis [58]; such endothelial damage can be compensated by endothelial repair, and circulating EPCs that originate in the BM are essential for this process [59]. Several studies report that circulating EPCs are reduced in the presence of classic cardiovascular risk factors, independent of the presence of established cardiovascular diseases (CVDs) [30]. For example, in patients with hypertension, or in smokers, the number of circulating EPCs is reduced [60], while EPCs from patients with diabetes mellitus type-2 are dysfunctional, and are characterized by decreased proliferative capacity, reduced adhesiveness, and a diminished ability to form capillary tubules *in vitro* [61]. The earliest anatomical sign of atherosclerotic remodeling is an increased thickness of the intima-media; this change is associated with a reduced number of CD34 + VEGFR2 + EPCs [62]. The later stages of coronary [63,64], carotid [65], cerebral [66] and peripheral atherosclerosis [67] are also characterized by a reduction in circulating EPCs.

In contrast, acute myocardial infarction and vascular trauma (modeled by coronary bypass grafting or burn injury) are associated with a rapid but transient increase of EPCs in the circulation [68,69], believed to be a compensatory mechanism to limit residual ischemia and achieve better reperfusion. *Ex vivo*, expanded EPCs from human PBMCs are able to augment limb salvage and myocardial function through neovascularization when transplanted into animal models of ischemic hind limb and acute myocardial infarction [70,71]. Some (but not all [40]) clinical studies [72–75] also report that autologous BM-derived cells, or *ex vivo* expanded autologous EPCs, have the ability to repair infarcted myocardium in humans [40].

Rheumatic diseases are associated with an increased incidence of CVDs [76,77] and are characterized by elevated endothelial dysfunction, which contributes to accelerated atherosclerosis [78]. Interestingly, the levels and functions of circulating EPCs are often reduced in patients with rheumatic diseases like psoriatic arthritis, rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis and anti-neutrophil cytoplasmic autoantibody-associated vasculitis [79]; such reduction is also seen in young patients without manifest CVDs, which implies that EPC dysfunction might be an early event in the pathophysiology of the atherosclerosis that is associated with rheumatic disease [79].

Based on the above evidence, EPC reduction is currently considered to be a mechanism whereby cardiovascular risk factors promote CVD, and a decrease in EPCs is also associated with the pathophysiology of rheumatologic disorders. In this context, restoring EPC function may prevent long-term cardiovascular complications associated with cardiovascular risk factors, and attenuate clinical manifestations of rheumatic diseases.

3. EPCs in solid tumors

While restoring EPC functions in CVD may prevent disease development, targeting EPCs in cancer might also have potential therapeutic benefits. EPC recruitment during tumor progression contributes significantly to the cancer-associated neovasculature — thus targeting these cells could be effectively used to halt tumor progression by preventing vascularization of neoplastic deposits. EPC-mediated vasculogenesis is a multistep process, which includes: a) mobilization of EPCs from BM; b) active arrest and trans-endothelial extravasation of EPCs into the interstitial space of the growing tumor; c) EPC incorporation of into neovessels or paracrine support of the nascent microvasculature.

Tumor-derived signals instigate the BM compartment to mobilize EPCs and recruit them to the tumor bed [80–82]. For example, the high levels of vascular endothelial growth factor (VEGF) produced by tumors mobilize BM resident EPC to enter the peripheral circulation, and enhance the recruitment of these cells the tumor sites [83,84]. VEGF mobilizes these cells by interacting with the VEGFR-1 and VEGFR-2 receptors (R) that are expressed on EPCs [85]. VEGF also induces MMP-9 expression by tumor cells, and results in the release of stem cell active soluble kit ligand, which in turn forces the translocation of quiescent VEGFR2 + c-kit + EPCs into the circulation for recruitment to sites of ischemia, inflammation or tumor growth [86,87].

Hypoxia also mobilizes EPCs [88]: malignant tumor growth results in neoplastic tissue hypoxia, which then mobilizes EPCs in a paracrine fashion. EPCs widely express CXCR4, which is the receptor for stromal cell-derived factor-1α (SDF-1α) and a member of the chemokine CXC subfamily [89]. Hypoxia induces SDF-1α secretion by stabilizing hypoxia-inducible factors (HIF) in tumor cells [90]; this mobilizes EPCs in the BM [91,92]. To date, both SDF-1α/CXCR4 and VEGFA/VEGFRs pathways are the principal known mediators of EPC BM mobilization during cancer development, and represent potential targets for new anti-vasculogenic therapies.

The contribution of EPCs to neovessel formation has been reported for several spontaneous, transplanted and metastatic solid tumor models [14–19,93–95]; however, other studies that use some of these same tumor models, report almost no incorporation of BM-derived EPCs to the nascent vasculature, and point instead to the possible pro-vasculogenic role of other BM-derived cell populations of hematopoietic origin [20–24,96].

From a functional point of view, an elegant study by Lyden et al. [14] used Id1/Id3 double KO mice to document a critical role of BM-derived EPCs in solid tumor progression: they showed that Id mutant mice fail to support growth in several transplanted syngenic tumor models because of impaired angiogenesis, which is associated with impaired BM mobilization and proliferation of VEGFR2 + EPCs and VEGFR1 + myeloid cells. Transplantation and engraftment of ß-galactosidase-positive wild-type BM, or VEGF-mobilized stem cells, into lethally irradiated Id mutant mice was sufficient to reconstitute tumor angiogenesis. Gao et al. [94] studied the involvement of EPCs in vessel formation in the Lewis lung metastatic carcinoma model (LLC), with use of tail vein injection of red-fluorescent protein (RFP +) LLC cells in mice that were reconstituted with BM from green fluorescent protein mice (BM-GFP$^+$) for tracking BM-derived cells. Dox-mediated inducible BM suppression of Id1 gene expression *in vivo* was used to assess a possible role of EPCs in mediating the micro- to macro-metastatic transition: this transition was indeed linked to the ability of EPCs to promote the angiogenic switch, which is necessary to achieve a large tumor size.

Accordingly, although inhibition of EPCs via Id1 silencing in BM cells was unable to reduce the number of micro-metastatic foci, it nevertheless prevented macro-metastatic transformation in this tumor model. Similar results were obtained with the use of a transgenic breast cancer mouse model (MMTV-PyMT), which spontaneously metastasizes to the lungs. Of note, the reduction in EPCs was specific, as no significant change was observed in the levels of BM-derived hematopoietic cells, including B cells, T cells, and myeloid or VEGFR1 + cells. In this study, the majority of BM-derived cells found in the tumors belonged to the hematopoietic lineage (CD11b +, CD45 RB +), and were localized around tumor vessels; however, up to 12% of the BM-GFP$^+$ cells were engrafted in the tumor-associated neovasculature; these cells express CD31, and localize in functionally active vessels, as shown by staining for isolectin B4 (GS-IB4) that was injected systemically in mice before sacrifice to identify perfused vessels. Lungs with micrometastases contained 5-fold more putative EPCs (defined as GFP + VE-cadherin + CD31 + CD11b − cells), compared to control mice without micrometastases. Interestingly, the loss of Id1/Id3 genes in genetically susceptible tumor models [95,97], and in chemically induced tumor models [98], had no effects on tumor onset or progression compared to Id1/Id3 wild-type control mice, questioning the biological importance of findings obtained by Lyden [14] and Gao [94] in the transplantable tumor models. The most plausible explanation of these conflicting results is that mechanisms regulating angiogenesis in transplantable models (*i.e.* xenograft models) differ from those in mouse models with spontaneously arising tumors; this underscores that the relative importance of EPCs to the process of tumor angiogenesis could vary, depending on the specific murine cancer-model used.

Transduction of mouse-derived BM progenitor cells with lentiviral vector (expressing genes from transcription-regulatory elements of the Tie2/Tek gene and GFP), followed by BM transplantation studies in several subcutaneous tumor models, led De Palma et al. [21] to conclude that BM-derived cells made almost no contribution to the vasculature of implanted tumor grafts in host mice. BM-derived cells recruited to the tumor bed were mostly macrophages and pericytes, with a peri-endothelial rather than in-vessel location. Gothert et al. [22] also reported that transplanted BM cells do not contribute to the endothelium of tumor vasculature, and that the majority of endothelial cells of tumor vessels are derived from the vascular endothelium of pre-existing tissue vessels.

Purhonen et al. [96] studied mobilization from the BM of VEGFR2 + EPCs in C57BL/6 mice that were implanted with B16 melanoma cells: they found that the mobilization of hematopoietic cells from the BM was correlated with the tumor growth rate; however, they observed no significant elevation in circulating BM-derived VEGFR2 + EPCs. Analysis of the tumor vasculature again revealed numerous peri-endothelial BM-derived hematopoietic cells, but no BM-derived VEGFR2 + cells were integrated into the vessels.

Overall, these studies show that 1) most controversies about the role of BM-derived cells in tumor angiogenesis concern the issue of whether or not BM-derived cells integrate into the endothelial layer of the tumor vessels; 2) a robust recruitment of BM-derived cells (mostly of hematopoietic origin, *i.e.* CD11b + or CD45 + cells) is consistently observed during tumor progression; these cells express some EPC markers, and their phenotype may overlap with that of putative EPCs. Collectively referred to as "vascular leukocytes", these cells include a variety of myeloid progenitor and precursor cells, monocytes, Tie-expressing macrophages, MDSCs, subsets of DCs, and neutrophils [26]: once recruited to tumor bed, they reside in close proximity to the vessels, and could be involved in promoting vessel formation, as showed by conditional targeting [20] and drug-targeting studies [99].

Thus, if we assume that some of these cells were included in the definition of EPCs, it is now possible to say that, in addition to vessel incorporation, tumor-recruited EPCs contribute to vessel formation by secreting pro-angiogenic growth factors [94] and independently by becoming incorporated in the nascent vasculature; these findings suggest that the paracrine role mediated by these cells is more important than their structural role.

Nevertheless, several additional points should be considered to explain why the different studies on the contribution of EPCs to tumor vessels come to such highly variable conclusions (reviewed by Gao, D. et al. [100]). It is possible that incorporation of EPCs into the tumor vasculature is tumor-stage specific, and the relative contribution of these cells to tumor-associated neovessels is greater at earlier rather than later stages of tumor growth [16]. In growing tumors, incorporated EPCs could eventually be replaced with non-BM-derived endothelial cells, after the angiogenic process has taken place [16]. Also, EPC-induced neovessel formation depends on the tumor type and its related stage-specificity [100]. Thus, the selection of specific mouse cancer models may be important for analyzing EPC-mediated neovascularization, which may take place at earlier or later stages, depending on the specific tumor models. Moreover, the ability of EPCs to mobilize varies among mouse strains [101]: the same tumor model could yield a higher or lower degree of BM EPC mobilization, based on the specific host mouse strain in which it was developed. And finally, several technical and experimental variables could also factor into the analysis: diverse strategies used to define murine EPCs (*i.e.* different markers used to define EPCs); techniques used to identify and measure EPC incorporation in the tumor blood vessels (*i.e.* immunofluorescence *versus* high resolution confocal microscopy, confocal techniques such as 3D computer rendering, immunohistochemistry, and use of light microscopy); type of system used to track BM-derived cells (GFP *versus* ß-galactosidase/LacZ + BM transplantation, evaluation of engraftment and percentage of engraftment after BM-transplantation procedure); functional *versus* total vessel staining by injecting (prior to sacrifice of tumor bearing mice) molecules that can specifically stain perfused vessels.

In the clinical setting, a higher level of circulating EPCs in the PB of cancer patients correlates with increased angiogenesis and metastases, and is associated with reduced patient survival, highlighting the importance of these preclinical findings. For example, the number of circulating EPCs is reportedly increased in patients with breast [45,102]; lung [103]; gastric [104]; gynecological [105,106]; liver [107]; and glioblastoma [108] cancers, compared to healthy individuals. And examination of secondary tumors in patients who were previously transplanted with HSCs from a sex-mismatched donor, has revealed the presence of donor-derived endothelial cells in the tumor-associated vasculature [17], suggesting that incorporation of BM-derived EPC into vessels may be a relevant biological phenomenon occurring in cancer patients.

In summary, although many issues still remain to be clarified, preclinical and clinical data consistently highlight the importance of EPCs (including subsets of BM-derived cells of hematopoietic origin) in mediating the progression of solid tumors, including metastatic dissemination.

3.1. Role of EPCs in mediating the development of resistance to anti-vessel therapies

Another area of growing interest concerns the potential role of EPCs in mediating the development of resistance to anti-cancer therapy, and more specifically to drugs that target tumor-associated vasculature. Shaked et al. [109] found that mobilization of BM-EPCs, induced by treatment with vascular disrupting agents (VDAs), mediates tumor re-emergence following the VDA therapy, by promoting the regrowth of vessels. Interestingly, inhibiting EPC mobilization with use of a VEGFR2-targeting anti-angiogenic drug, or in Id1/Id3 knockout transgenic mice (that are unable to mobilize EPCs) enhances VDA anti-tumor activity, suggesting that EPCs mediate resistance to VDA therapy. Taylor et al. [110] recently reported that a single dose of CA-4-P (another VDA) induces two – rather than one – EPC peaks in the PB of treated mice: the first peak occurs a few hours after administration of the VDA, as previously reported by Shaked et al. [109], while the second (previously unrecognized) peak appears 3 to 4 days after the

administration of VDA. Similar EPC kinetics was observed in cancer patients treated with ombrabulin, also a VDA drug. Use of a VEGFR2 targeting anti-angiogenic drug to inhibit the second (but not the first) peak of EPCs dramatically enhances therapeutic activity of VDA treatment. These studies suggest that circulating EPCs may represent important biomarkers of drug activity and/or resistance in patients as well as mouse tumor models, and offer a new therapeutic target for enhancing the activity of VDA drugs. However, as discussed in a commentary by De Palma et al. [111] on the latter study [110], anti-angiogenic treatment with a drug that targets VEGFR-2 not only suppresses the late EPC spike and the consequent incorporation of EPCs in the tumor blood vessels, but also dramatically reduces tumor infiltration by other BM-derived hematopoietic cells that may phenotypically overlap with EPCs. In line with this observation, BM mobilization and tumor recruitment of Tie-expressing macrophages have recently been shown to mediate resistance to VDA therapy [112], favoring the hypothesis that EPCs as well as other BM-derived cells are important in this process.

Some conventional chemo-drugs like taxanes, but not others like gemcitabine, induce a rapid mobilization of EPCs when administered at maximum tolerated doses, in mice [113] and in patients [114]; and in preclinical models, inhibition of EPC BM mobilization, induced by the administration of the paclitaxel, with a VEGFR2-targeting drug resulted in increased anti-tumor activity, similar to what has been described with VDA agents [113]. Note that paclitaxel, but not gemcitabine, is endowed with vessel targeting properties [115], suggesting that the rapid mobilization of EPCs from the BM may be a consequence of some chemo-agents having the capacity to disrupt the tumor vasculature, thereby creating the need for a rapid host response to repair the damaged.

When certain chemotherapy drugs (*i.e.* cyclophosphamide) are administered in a "metronomic" fashion (frequent, repetitive low doses) rather than at the maximum tolerated dose, they can mediate an anti-angiogenic effect [116]; this is because metronomic administration of a chemo-agent blocks the rapid EPC mobilization response (seen when the same drugs are administered at maximum tolerated dose), further supporting the notion that EPC mobilization serves as a mechanism of resistance to some conventional chemo-agents. In fact, use of metronomic therapy may offer a therapeutic strategy to specifically target EPCs [117], partially explaining why low-dose metronomic chemotherapy exerts potent anti-angiogenic effects.

Finally, EPCs and other BM-derived hematopoietic cells are implicated in the development of resistance to pure anti-angiogenic agents (e.g., VEGFR2-targeting drugs) [118]. Like VDAs, high dose anti-angiogenic drugs can cause vessel regression, an acute increase in intratumoral hypoxia, and in some cases, in activation of HIF-1 alpha in tumor cells [118]. Hypoxia and activation HIF-1alpha promote secretion of SDF-1alpha and VEGF, which may stimulate the mobilization and recruitment of EPCs (and other BM-derived hematopoietic cells), following anti-angiogenic treatment [119,120]. Once recruited, the EPCs can promote tumor growth by eliciting new blood vessel formation [109]. Reduction of EPC mobilization has been observed in cancer patients during treatment with VEGF inhibitors [121,122]; however, circulating EPCs increase during drug-free intervals [121] — this may promote tumor vessel regrowth and lead to the development of resistance to these agents. Note that, as for EPCs, other pro-vasculogenic BM-derived cells (e.g., Gr1 + CD11b MDSCs [123], macrophages [124], and VEGFR-1 + myeloid cells [125]), whose phenotype overlaps with that of EPCs, are also recruited following anti-angiogenic treatment. Again, the specific contribution of EPCs, *versus* other BM-derived hematopoietic cell populations, to the development of resistance to anti-angiogenic drugs is not understood.

4. EPCs in hematological malignancies

A major challenge in the study of vasculogenesis in hematological cancers is that the hematopoietic and endothelial lineages originate from a common mesodermal progenitor, the hemangioblast [48], and adult HSCs and hematopoietic progenitor cells may also exhibit hemangioblast-like activity [52–54]. Thus, EPCs in hematological malignancies may be related to the neoplastic clone, with both sharing a common ontogeny (Fig. 2). For example, acute myeloid leukemia (AML) cells arise from a malignant hematopoietic stem and progenitor cells, and it is possible that a leukemia hemangioblast is responsible for generating malignant leukocytes as well as ECs. Accordingly, specific genetic aberrations are observed in the tumor vasculature and in tumor-derived ECs in leukemia [126], lymphoma [127], and multiple myeloma (MM) [128]. Streubeland and co-workers [127] looked for known cytogenic alterations in the ECs of 27 B-cell lymphomas, and found that, on average, 37% of the microvascular ECs harbor lymphoma-specific chromosomal translocations; these findings suggest that microvascular ECs in B-cell lymphomas are, in part, tumor related, and raise the provocative possibility that a proportion of the ECs in lymphomas are derived directly from the lymphoma clone, or from a common, diseased HSC/progenitor in the BM.

A subpopulation of vascular progenitor cells (VEGFR2 + CD31 − CD34 −) that harbor the BCR/ABL gene fusion has been identified in the BM of patients with chronic myeloid leukemia (CML) [129]: single cells of this subpopulation reportedly have the potential to form malignant hematopoietic cells and ECs *in vitro*. When transplanted into NOD/*scid* mice, these VEGFR2 + CD31 − CD34 − cells reproducibly transferred CML to recipient mice, and generated BCR/ABL-expressing ECs within blood vessels [129]. Furthermore, Gensilius and co-workers utilized cultured CFU-ECs from the PB of patients with CML and found that these cells displayed BCR/ABL translocation [126]. In other studies, transformed genotypes, including BCR/ABL and the Janus kinase 2 (JAK2) V617F mutation, were present in PBMC derived-CFU-Hill colonies, but not in ECFCs [33,130].

Patients with chronic idiopathic myelofibrosis, polycythemia vera, and essential thrombocytopenia present with higher levels of circulating cells that co-express the putative EPC markers CD34, CD133, and VEGFR2 [131], thereby indicating the presence of endothelial precursors among the circulating progenitor cells. Subsequent clonogenic stem cell assays, undertaken to confirm differentiation towards the hematopoietic and the endothelial cell lineages, revealed trisomy 8 and JAK2 V617F markers in the grown endothelial cells of patients who were positive for trisomy 8 or harbored the JAK2 V617F variant in the PB, again pointing to a common clonal origin of the hematopoietic and endothelial cell lineages [131], and corroborating a previous report from Gunsilius and co-workers [126]. And in a parallel clonal analysis of ECFCs and CFU-ECs in 11 patients with polycythemia vera [33], Yoder and co-workers documented presence of the JAK2 V617F variant (used to determine the clonal relationship of ECFCs and CFU-ECs) in the CFU-ECs from all 11 patients; in contrast, ECFCs from 10 of the same 11 patients did not carry this mutation, suggesting that ECFCs are not hematopoietic in origin. In one patient, however, three ECFC colonies did display the mutant JAK2 allele: the authors explained this discrepancy as the result of a possible contamination of the ECFCs by hematopoietic mononuclear cells [33]. Additional clonal analysis in a larger cohort of patients may be required to draw definitive conclusions regarding the clonal relationship between HSCs, CFU-ECs and ECFCs [33].

Rigolin and co-workers studied 5 MM patients with a 13q14 deletion, and found that CECs, from these individuals carried the same chromosome aberration as did plasma cells (11–32% of CECs with del13q14); these cells also expressed CD133, and thus were considered as having immunophenotypic features of EPCs. In contrast, CECs from 3 patients with monoclonal gammopathy of undetermined significance (MGUS) and del13q14 were cytogenetically normal and had a mature immunophenotype. Moreover, immunoglobulin genes were found to be clonally rearranged in CECs from MM patients. This observation suggests that CECs originate in a common hemangioblast precursor that generates both plasma cells and ECs. It also indicates a direct

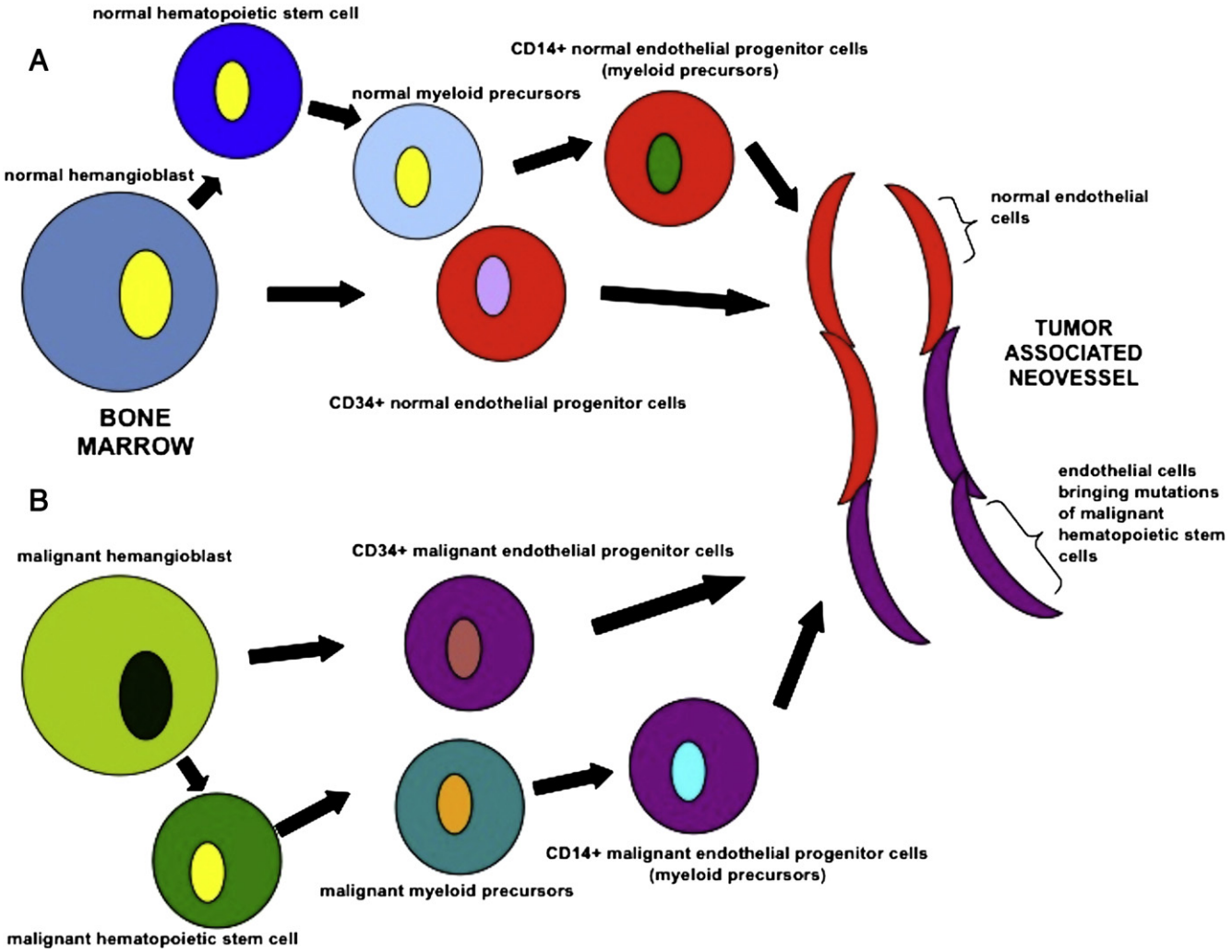

Fig. 2. Endothelial progenitor cell-mediated vasculogenesis in hematological malignancies. Schematic overview of possible endothelial progenitor cell-mediated neovessel formation in hematologic cancers. The hematopoietic and endothelial lineages are generated from a common mesodermal progenitor, the hemangioblast (normal hemangioblast). Perhaps in blood cancers the hemangioblast is related to the neoplastic clone with which it shares ontogeny (malignant hemangioblast). The malignant hemangioblast is then able to generate directly malignant EPCs (CD34+ endothelial progenitor cells) and indirectly, malignant EPCs with myeloid features (CD14+ malignant endothelial progenitor cells). CD14+ EPCs originate from hematopoietic stem cell and myeloid cell lineage and co-express markers from the myeloid cell and endothelial cell lineages. CD34+ progenitor cells originate directly from the hemangioblast and exclusively express markers from the EC lineage. Both normal and malignant EPCs integrate in the tumor-associated neovessel through the process of vasculogenesis.

contribution of MM-derived CECs to tumor vasculogenesis and to disease spread and progression [128].

Braunsteinand and co-workers [132] used a human androgen receptor assay (HUMARA) to determine patterns of X-chromosome inactivation in female patients (n = 11), in order to uncover a possible clonal origin of EPCs; they also explored whether EPCs and BM cells displayed the clonotypic immunoglobulin heavy-chain gene rearrangement, which indicates clonality in B cells. Of note, EPCs in this study were obtained by plating PBMCs on laminin-coated wells, and were defined as late outgrowth colonies (*i.e.* ECFCs), even though their morphology was more suggestive of EC-CFUs than of ECFCs. In 64% (n = 7) of the patients, HUMARA analysis of EPCs revealed significant skewing (≥77% expression of a single allele), while in 4 patients, the X-chromosome inactivation skewing was extreme (≥90% expression of a single allele). Furthermore, PCR amplification with variable heavy-chain primers resulted in amplification of the same product in EPCs and BM cells in 71% (n = 5) of 7 patients, while no immunoglobulin heavy-chain rearrangement was found in EPCs from healthy controls [132]. This again suggests that in MM patients, EPCs may be related to the neoplastic clone, and this may represent an important mechanism for upregulation of tumor neovascularization.

Collectively, the above reports point to the existence of an adult hemangioblast population within the setting of hematological malignancies, and indicate that vessel formation in these cancers is driven by an alternative path (Fig. 2). They also suggest that tumor-associated ECs that harbor cytogenetic mutations are likely not derived from putative EPCs (defined by their ability to form ECFC colonies), but rather from a population of hematopoietic-derived cells that have the ability to form EC-CFU colonies. The existence of a bi-potential malignant HSC could reveal a source of relapsed disease and represent a new target for therapy.

4.1. Correlation of the number of EPCs with tumor burden in hematological malignancies

4.1.1. Non-Hodgkin's lymphoma

The significance of neovessel formation in lymphomas varies greatly among different studies, probably due to the heterogeneity of lymphoma subtypes and the different techniques used for microvessel staining and scoring. In general, vessel formation is higher in aggressive subtypes, including Burkitt's and peripheral T-cell lymphomas, compared to the more moderate levels seen in diffuse large B-cell lymphomas and

lower levels reported for indolent follicular lymphomas [133]. Not much is known about the role of vasculogenesis in these diseases. A preclinical study reported a correlation between the level of EPCs in the PB and tumor volume in SCID mice bearing human lymphoma [134]. Igrejaand and co-workers studied CD133 + CD34 + VEGFR2 + EPCs in the PB and lymph nodes of patients with NHL, and found that the highest levels of circulating EPCs were present in younger NHL patients and in those with aggressive disease, whereas complete response to treatment led to a significant reduction in this cell type. EPCs were also detected in vascular structures and in the stroma of pathological lymph nodes of these patients, and their presence was correlated with lesion size and increased angiogenesis [135]. The presence of circulating EPCs in NHL patients suggests involvement of these cells and of vasculogenesis in the pathogenesis of lymphomas.

4.1.2. Leukemias

A 3-fold increase in BM microvessel density (MVD) has been described in patients with B-cell chronic lymphocytic leukemia (CLL), compared to healthy controls, indicating a key role of CLL-associated neovessels in the pathogenesis of the disease [136]. Rigolin and co-workers [137] used flow cytometry to study levels of CECs and putative EPCs in 170 patients with CLL. CECs were defined as circulating CD45 − CD34 + VEGFR2 + cells, while EPCs were defined as CD45 − CD34 + VEGFR2 + and CD133 + cells. The numbers of CECs and EPCs were increased relative to controls; higher levels of CECs and EPCs identified a subset of patients with more aggressive disease. Interestingly, FISH analysis of sorted cells showed that, in some patients, CECs present with the same cytogenetic lesion of neoplastic lymphocytes and the same immunophenotypic features as for EPCs. The gene expression profiling of sorted CECs revealed a molecular pattern that suggested a derivation from CLL cells with increased survival and proliferation, diminished adhesion to extracellular matrix, and enhanced pro-angiogenic function, compared to their counterparts from normal subjects [137]. These findings suggest that EPCs may incorporate into tumor-associated vessels in CLL, and thereby make a substantial contribution to vessel formation.

Judah Folkman's group first described increases in BM vascularization in leukemia patients: specifically, they demonstrated an increased blood vessel content in the BM of acute lymphoblastic leukemia (ALL) patients compared to the normal counterparts [138]. Hussong et al. found significantly increased MVD in the BM of acute myeloid leukemia (AML) patients compared to healthy controls, suggesting a role for vessel recruitment in AML pathogenesis [139]. Importantly, increased BM vasculature showed a strong positive correlation with overall survival of leukemia patients [140,141]. The role of EPCs in acute leukemia remains poorly investigated, especially at the preclinical levels. Wierzbowska and co-workers counted resting (r) CECs, activated (a) CECs, and EPCs [142] in the PB of AML patients and healthy subjects, and correlated these numbers to disease status, known prognostic factors and response to treatment. EPCs were defined as negative for the hematopoietic marker CD45, and positive for EC markers CD34, CD31 and for the EPC marker CD133. Resting CECs were defined as CD45 − CD133 − CD31 + CD34 + CD146 + and negative for activation markers (CD105, CD106). CD105 + or CD106 + mature ECs were classified as (a) CECs. The numbers of (a) CECs, (r) CECs and EPCs were significantly higher in AML patients than in controls; the CEC count was significantly higher in AML patients with white blood cell counts (WBC) of $>15 \times 10^9$/L, with elevated lactic dehydrogenase (LDH) levels, and with a higher (more than median) absolute blast count in PB, than in the group with WBCs of $<15 \times 10^9$/L, normal LDH level and lower absolute blast count. The levels of (a) CECs, (r) CECs and EPCs in patients who achieved complete remission after the first course of chemotherapy, were substantially lower than at diagnosis, while they did not change in patients who were refractory to treatment. In a subsequent study [143], the kinetics of mature CECs, both (a) CECs and (r) CECs, as well as EPCs and apoptotic CECs (CECs[AnnV +]), was evaluated in AML patients treated with standard chemotherapy. Significantly higher numbers of (a) CECs, (r) CECs, EPCs, and CECs [AnnV +] were detected in AML patients than in healthy controls. The elevated EPCs and absolute counts in PB, and the low CECs [AnnV +] were associated with a higher probability of treatment failure. (a) CEC, (r) CEC, EPC, and CEC [AnnV +] counts were significantly lower in complete remission than at diagnosis. A significant decrease in the CEC count and an increase in the numbers of CEC [AnnV +] were also observed 24 h after the first dose of chemotherapy in complete remission patients. And in refractory AML, the (a) CEC, (r) CEC, EPC, and CEC [AnnV +] counts, assessed before and after induction chemotherapy, did not differ significantly; a significant decrease in the CEC count and an increase in CEC [AnnV +] number, was noted only after the last dose of chemotherapy [143]. These reports point to a need to further investigate the role of vasculogenesis in acute leukemias.

4.1.3. Myelodysplastic syndromes

Relative to control subjects, BM MVD is significantly increased in myelodysplastic syndromes (MDS) [144], and lenalidomide, a drug endowed with potent anti-angiogenic activity, is an approved therapeutic option for these patients (5q-variant). Della Porta and co-workers [145] studied CECs in 128 patients with MDS. CEC identification was based on CD45 staining to exclude hematopoietic cells; CECs were then identified in the CD45 − gate, as CD146 + CD34 + cells, while CD133 expression was used to discriminate between endothelial progenitors and mature ECs, and expressed as progenitor-to-mature ratio. MDS patients had higher CEC levels than controls, and the number of CECs was inversely related to the international prognostic scoring system risk level. On the other hand, BM microvessel density was positively correlated to CECs, with low-risk patients showing the strongest association. The progenitor-to-mature CEC ratio was higher in MDS patients than in healthy subjects, and the highest values were found at diagnosis, indicating that the majority of CECs in MDS, but not in healthy individuals, consists of precursor cells (*i.e.* EPCs), with the ability to differentiate into endothelia. In fact, as assessed by flow cytometry, CEC levels are positively correlated with their ability to produce ECFCs *in vitro*, a number that is significantly higher in MDS patients than in the healthy population. FISH analysis also showed that a variable proportion of CECs (from 40 to 84%) carries the same chromosomal aberration as does the neoplastic clone, while ECs isolated from *in vitro* assays are negative for the chromosomal abnormality [145]. These results suggest that EPC-mediated vasculogenesis represents an important target in MDS.

4.1.4. Multiple myeloma

The role of vasculogenesis in this disease also remains poorly investigated. An increased MVD in the BM of active MM patients (compared to those with smoldering MM and MGUS) was first described by Vacca et.al [146]. Rajkumar et al. then showed a gradual increase of BM angiogenesis along the disease spectrum of plasma cell dyscrasias, from MGUS to smoldering MM, newly diagnosed MM, and relapsed MM [147]. Zhang et al. used RT-PCR to evaluate the role of EPCs (identified as cells co-expressing CD133 and VEGFR2 by RT-PCR in total PBMCs) and CECs (identified flow cytometry as CD34 + CD146 + CD105 + CD11b − cells) in 31 consecutive MM [148] and showed that levels of CECs and EPCs in PB are higher in patients than in healthy controls. CECs were 6-fold higher in patients compared to controls, and correlated positively with serum M protein and beta(2)-microglobulin, both of which are markers of disease activity. Moreover, elevated levels of CECs and EPCs co-varied with disease activity and response to thalidomide.

In another study [149], EPC numbers were assessed in 75 patients with MM, and the numbers were correlated with clinical and laboratory parameters as determined in the PB from: higher numbers of EPCs (defined as CD45 −/dim CD34 + CD133 + CD31 + VEGFR-2 + cells) were observed in MM patients compared to healthy subjects; these numbers increased progressively from Durie & Salmon stage I to stage

Table 3
Summary of clinical studies reporting circulating EPC alterations in several types of hematological malignancies.

Disease (Reference)	Finding/circulating EPC phenotype	Correlation/observation
Non-Hodgkin's Lymphoma (NHL) [135]	- Increased CD133 + CD34 + VEGFR2 + EPCs	-Highest EPC levels found in younger lymphoma patients and in those with aggressive disease. -Significant EPC reduction following complete response to treatment.
[155]	-Increased CD133 + CD34 + VEGFR2 + EPCs	-Higher EPC number in NHL patients than controls. -Higher EPC levels in patients with stage III-IV than stage I-II NHL.
Chronic Lymphocytic Leukemia (CLL) [137]	-Increased CD133 + CD34 + VEGFR2 + CD45 − EPCs	-Higher level of CECs (with immunophenotypic features of EPCs) identified a subset of patients with a more aggressive disease course characterized by a shorter time to first treatment.
Myelodysplastic Syndromes (MDS) [145]	-Increased CEC levels characterized by higher progenitor-to-mature CEC (CD45 − CD146 + CD34 + cells) ratio based on CD133 positivity - Increased ability to form ECFCs *in vitro*	-Inverse relationship was found between CECs (with higher progenitor-to-mature ratio) and IPSS risk. -Positive correlation between marrow microvessel density and CECs, low-risk patients showing the strongest association. - Progenitor-to-mature CEC ratio higher in MDS patients than in healthy subjects.
[156]	-Increased CD133 + CD34 + CD31 + CD45 − EPCs	-No association was found between EPC levels and FAB or IPSS subtypes or survival.
Chronic Myeloproliferative Diseases (CMDs) [131]	-Increased CD34 + VEGFR2 + CD133 + EPCs in a subset of chronic idiopathic myelofibrosis patients	-Molecular markers trisomy 8 and JAK2 V617F mutation were found in the grown endothelial cells of patients positive for trisomy 8 or JAK2 V617F in the peripheral blood.
[157]	- Increased CD34 + VEGFR2 + CD133 + EPCs especially in chronic idiopathic myelofibrosis patients - Increased CD34 + VEGFR2 + CD133 + EPCs in Ph-negative CMDs compared to healthy controls	- EPCs were associated with younger age and a diagnosis of prefibrotic stage of myelofibrosis -In idiopathic myelofibrosis patients the numbers of EPCs detected was greater than the highest value found in patients with other Ph-negative CMDs.
Acute leukemia (AL) [142]	-Increased CD34 + CD31 + VEGFR2 + CD133 + CD45 − EPCs	- EPC levels after the first course of chemotherapy were significantly lower than at diagnosis in the patients who achieved complete remission, and didn't change in patients refractory to treatment.
[143]	-Increased CD34 + CD31 + VEGFR2 + CD133 + CD45 − EPCs	- Elevated EPC count associated with higher probability of induction treatment failure.
Multiple myeloma (MM) [150]	- Increased CD34 + CD133 + VEGFR2 + early EPCs, CD34 + VEGFR2 + mature EPCs and VEGFR2 +-cells in bone marrow of MM patients compared to MGUS patients and healthy controls.	- MM patients present the highest level of early and mature EPCs and VEGFR2 +-cells in the BM, particularly with advanced and active disease
[149]	- Increased numbers of CD34 + CD133 + CD31 + VEGFR2 + CD45 + dim EPCs	- EPC levels increased progressively from D&S stage I to stage III - No correlation was established between baseline EPC numbers and PFS and OS - Significant decline in EPC numbers after therapy was observed in patients who attained at least a partial response -EPC levels correlated with treatment response duration

EPCs: endothelial progenitor cells. CECs: circulating endothelial cells. ECFCs: endothelial colony-forming cells. IPSS: international prognostic scoring system. FAB: French–American–British classification. MGUS: monoclonal gammopathy of undetermined significance. D&S: Durie and Salmon stage.

III, while no correlation was established between baseline EPC numbers and progression-free survival or overall survival. However a significant decline in EPC numbers was observed after therapy in patients who attained at least a partial response: EPC levels in PB correlated with response duration, at a baseline cut-off value of 19.6 EPCs/uL and 6.5 EPCs/uL after therapy [149].

Udi and co-workers [150] evaluated the levels of early EPCs (CD34 + CD133 +, VEGFR2 +), mature EPCs (CD34 + VEGFR2 +) and VEGFR2 + cells in the BM (the natural source of EPCs) of MM *versus* MGUS patients and healthy controls. MM patients demonstrated the highest early and mature EPCs and VEGFR2 + cells in the BM, particularly with advanced and active disease [150].

We recently showed that MM patients, including those in the smoldering/early phase of disease, display a significant increase in circulating CD34 + VEGFR2 + cells, and in colony-forming ability of both EC-CFUs as well as ECFCs, compared to healthy controls. In several murine MM models (both orthotopic xenograft and singenic models) we found an early mobilization of BM-derived CD34 + VEGFR2 + cells after injection of tumor cells. These results suggest that in MM, vasculogenesis may represent an early pathogenic event that contributes to the "angiogenic switch", which takes place during the transition from early MGUS/smoldering MM to late overt/active MM phase of disease [151].

In summary these studies show that EPCs are associated with disease stage, prognosis and response to treatment of hematologic malignancies (Table 3); thus they are likely to contribute to the progression of these diseases. However, a number of different approaches have been used by investigators for the quantification of EPCs, and this makes reported results difficult to compare. For this reason the role of EPCs in the pathogenesis of hematologic malignancies has still not been clarified.

5. Conclusion

Tumor-associated neovasculature offers an important therapeutic target; however the clinical efficacy of anti-vessel treatment, although significant, is transient; most patients soon develop resistance to therapy, and the tumors inevitably progress. Anti-vascular drugs developed to date are essentially endowed with solely anti-angiogenic activity; however, it is becoming increasingly evident that several mechanisms, other than just angiogenesis, contribute to neovessel formation — these include vascular co-option [152], mosaicism, vasculogenic mimicry [153,154], and postnatal vasculogenesis [13] all of which been deeply investigated in solid malignancies, but whose roles in blood tumors are less well understood. We now know that these processes participate in the development of resistance to anti-angiogenic drugs [118] and they could represent targets for designing new anti-vascular molecules in solid as well as hematological malignancies.

Of the processes listed above, BM-derived EPC-mediated vasculogenesis may represent an important event, especially at the early stage of solid tumor growth, when EPCs make a critical contribution to promoting the "angiogenic switch", and also at the metastatic phase of the disease, when EPCs are essential mediators of the micro- to macro-metastatic transition [16,94]. Analyses of hematologic malignancies suggest that the ontogeny of EPCs could be related to that of the neoplastic clone; thus, characterization of tumor-associated EPCs in hematological malignancies may provide clues for more specific anti-vascular therapy that has a direct anti-tumor effect and an indirect effect related to simultaneous anti-vascular activity. Thus, therapeutic strategies aimed at EPCs should also be considered in the development of treatments for blood cancers.

The full impact of vasculogenesis in the pathogenesis of hematological cancers has yet to be clarified, primarily due to technical reasons including the lack of a consensus definition of EPCs themselves and lack of standardized methodologies for the quantification of these cells. Indeed, recent studies have established that most of the cells previously identified as circulating EPCs likely represent pro-vasculogenic hematopoietic cells at various stages of differentiation; this may account for many of the biological activities actually attributed to putative EPCs.

Acknowledgements

Support was provided in part by NIH grant R01CA154648, the Leukemia and Lymphoma Society (LLS), and the European Commission's Seventh Framework programme (EU FPT7) OVER-MyR (number 278706 to AV). We thank Sonal Jhaveri (PGSAO, Dana Farber Cancer Institute) for editing the manuscript.

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