The Role of Endothelial Progenitor Cells in Tumour Vasculogenesis

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Abstract

Objective: To review the biological behaviour of endothelial progenitor cells and their role and significance in tumour vasculogenesis. Data Sources: The data in this review were mainly from Medline and PubMed for the relevant articles in English published from March, 1997, to March, 2008. The search terms were ‘endothelial progenitor cells’ (EPCs) and ‘neoplasm’. Articles about the biological behaviour of EPCs and their roles in tumour vasculogenesis were included. Results: EPCs, whose characteristics are similar to those of endothelial cells (ECs) and stem cells, contribute to tumour vasculogenesis during tumour progression. The mobilisation, recruitment, homing and incorporation of EPCs into tumours are multi-step and multi-factor events during tumour vasculogenesis. This complex process requires the participation of many growth factors and cells, such as tumour cells, ECs, stromal cells and EPCs in the tumour microenvironment. However, there is still some debate about EPC distribution, contribution, origin and differentiation in tumour vasculogenesis. Conclusions: The characterisation of tumour-associated EPCs may provide valuable clues for more specific anti-angiogenesis therapy and/or tumour diagnosis. Many challenges remain in understanding definition, differentiation, mobilisation and recruitment of EPCs.

Introduction

Tumours are endowed with angiogenic-inducing capability, and their growth, invasion and metastasis are angiogenesis dependent. More recent data suggest that the development of the tumour vasculature occurs through two complementary processes: angiogenesis (sprouting from pre-existing blood vessels) and vasculogenesis (spontaneous development of vessels through mobilisation, recruitment, differentiation and vascular incorporation of bone marrow (BM)-derived endothelial progenitor cells (EPCs) [1]. Over the past several years, a substantial amount of evidence from animal as well as human studies has advanced our knowledge of how EPCs contribute to tumour vasculogenesis [2, 3]. These investigations support the hypothesis that EPCs can be considered as prognostic markers, even as targeting vectors for cancers [4, 5]. However, the role of EPCs in tumour origin, distribution and contribution remains to be elucidated [6, 7]. Here we have critically discussed some controversial questions.
The Biology of Endothelial Progenitor Cells

Following the landmark publication in 1963, since then, with a greater understanding of angiogenesis, many others have confirmed that stem cells in the adult circulation could contribute to growth of new endothelium in blood vessels [8]. Two decades ago, Asahara et al. [9] were the first to demonstrate the existence of circulating EPCs (CEPCs) in adult peripheral blood. Subsequently, several researchers have successfully separated EPCs from BM [10], cord blood [11], somatic muscles [12] and vascular parenchyma [13]. EPCs have two characteristics: firstly, similar to endothelial cells (ECs), for example, they have the ability to migrate, form primitive tubes and adhere to substratum [14], and secondly, similar to embryonic angioblasts, they show a high proliferation state, differentiate and have colony-forming capacity. So far, culture assays are the best approach to define EPCs. Most authors are in agreement that AC133, together with CD34 and VEGFR-2, is currently the ideal selective marker for identifying EPCs. Recently, it has been confirmed that risk factors of vascular diseases, such as age, smoking and diabetes, can reduce the concentration and activity of EPCs in peripheral blood and constraint angiogenesis capability accordingly [15, 16]. Lower numbers of EPCs appear to be correlated with an impaired capacity for endothelial repair that could affect cardiovascular disease progression [17]. Therefore, autologous, immunologically neutral EPCs constitute a convenient source for the construction of living blood vessel and heart valve replacements as well as the endothelialisation of intravascular stent devices for many applications [18]. Interestingly, evidence has emerged that EPCs contribute to tumour-induced new vessel growth, which is discussed in detail later in this review.

Contribution of EPCs to Tumour Vasculogenesis: Animal and Clinical Investigations

The following evidence supports the idea that EPCs contribute to tumour vasculogenesis: (a) a number of labelled EPCs are incorporated into tumour neovessels in vivo; (b) EPCs have the capacity to restore impaired angiogenesis; (c) EPCs have essential roles in tumour initiation, progression and metastasis, and (d) EPCs in the circulation and tumour tissues are of clinical significance.

I Incorporation of Labelled EPCs into Tumour Neovessels in vivo

Genetically labeled EPCs, expressing the β-galactosidase, green fluorescence protein (GFP) or thymidine kinase genes, were found to migrate and become incorporated into the angiogenic vasculature in growing tumours when transplanted into sub-lethally irradiated tumour-bearing mice. Asahara et al. [1] engrafted tumour-bearing mice with transgenic BM cells, in which constitutive LacZ expression was under the transcriptional regulation of fetal liver kinase-1 (Flk-1) and Tie-2 (endothelial-specific promoters). Histological examination of the tumour tissues showed localisation of Flk-1- and Tie-2-expressing cells of the endothelial lineage in blood vessels and stroma around the vasculature. In murine intracranial glioma models, the recruitment of GFP-marked EPCs into growing brain tumour and their incorporation into the vascular network occurred during the period of increasing vascular density and preceded the expansion of the tumour [19].

EPCs Have the Capacity to Restore Impaired Angiogenesis

Ischaemic diseases improve after EPC transplantation. EPCs have also been shown to restore impaired tumour vasculogenesis [20]. Although adult mice with reduced Id gene dosage cannot support tumour-induced angiogenesis, both B6RV2 lymphoma and Lewis lung carcinoma cells are able to form fully vascularised tumours in Id-deficient mice that have received wild-type BM transplants [2]. In addition, BM-derived cells, including EPCs, restored impaired VEGF-driven angiogenesis in mice lacking placental growth factor [21].

The Essential Role of EPCs in Tumour Progression

Some other studies were able to identify a time during tumour development at which the contribution of CEPCs to cancer vessels was apparently more relevant. A mathematical model showed that the percentage of EPC-derived vascular density increases from approximately 2% ($R_t = 0.5$ mm) to 25% ($R_t = 1.0$ mm). As the tumour gets larger ($R_t = 1.5$ mm), the fraction of EPC-derived vascular volume increases to approximately 40%, in agreement with the number of EPCs identified in certain tumours [2]. Moreover, Spring et al. [22] transplanted GFP-ex-
pressing BM cells into RIP1-Tag5 mice lethally irradiated to monitor the recruitment of EPCs during distinct phases of vessel remodelling. The frequency of GFP+ ECs increases with tumour progression, being 5.8 ± 0.8, 14.3 ± 1.7 and 26.8 ± 4.1% at 12, 14 and 16 (late-stage tumours) weeks, respectively.

Disseminated malignant primary tumour cells colonise target secondary organs, form dormant micrometastases, activate the angiogenic switch and progress to macrometastases [23]. Gao et al. [24] implanted Lewis lung carcinoma cells stably expressing red fluorescent protein into syngeneic mice reconstituted with BM cells expressing GFP. As expected, many BM-derived GFP+ cells were recruited into both micro- and macrometastases. The total number of metastases increased with time (average 22 and 35 per animal on days 21 and 28, respectively), with a concomitant increase in macrometastases (1 mm in diameter, 47% on day 28), which indicated a time window of micrometastasis to macrometastasis progression. Fluorescence-activated cell sorting analysis showed that the luminally incorporated BM-EPCs represent on average 12.7% of total ECs. These findings establish the role of EPCs in metastatic progression in preclinical models and suggest that selective targeting of EPCs may merit investigation as a therapy for cancer patients with lung metastases.

Therefore, it is concluded that EPCs play an increasingly important role in tumour progression.

Table 1. Clinical significance of EPCs to tumour activity

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>EPC levels</th>
<th>Correlated with EPC levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematological malignancies</td>
<td>circulating VE-cadherin RNA</td>
<td>response to treatment, disease activity</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>circulating and biopsy-derived EPCs</td>
<td>response to treatment, disease activity</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>CEPCs</td>
<td>response to treatment, disease activity</td>
</tr>
<tr>
<td>Acute myeloid leukaemia</td>
<td>circulating endothelial-like cells</td>
<td>disease activity [25, 35]</td>
</tr>
<tr>
<td>Inflammatory breast cancer</td>
<td>tumour-infiltrating EPCs</td>
<td>disease activity [26]</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>circulating Tie-2 mRNA, CEPCs</td>
<td>tumor size [61], disease activity [27], pro-angiogenic factors, response to surgical treatment or chemotherapy [26] poor overall survival, response to treatment [29], disease activity [30]</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>CEPCs</td>
<td>curative surgery, recurrence time [28], VEGF, PDGF, prognostic markers [3] cumulative survival [31]</td>
</tr>
<tr>
<td>HCC</td>
<td>CFU scores, CEPCs and tissue-derived EPCs</td>
<td>response to treatment, disease activity</td>
</tr>
<tr>
<td>Cancer with bone metastases</td>
<td>circulating AC133 mRNA</td>
<td>response to treatment, disease activity</td>
</tr>
</tbody>
</table>

CFU = Colony-forming units.

The Clinical Significance of EPCs in the Circulation and Tumour Tissue

EPCs have been detected with increased frequency in the circulation and tumour tissue in patients with multiple myeloma [25] and inflammatory breast cancer [26] (table 1). Their presence correlated with disease activity, and it was of prognostic value in patients with tumours [27, 28]. Cell surface markers of EPCs (AC133) were used to validate the incorporation of EPCs into neovessels of some tumours, such as inflammatory breast cancer [26], non-small cell lung cancer [29, 30] and hepatocellular carcinoma (HCC) [3]. In the circulation, there are significantly higher colony-forming unit scores in patients with HCC compared with those with cirrhosis or healthy controls [28]. In addition, AC133, but not CD146, mRNA expression is greater in cancer patients with metastatic disease, specifically with bone metastasis, which seems to be an independent prognostic factor for overall survival [31]. Furthermore, AC133 and other EPC-specific markers, e.g. Tie-2 and VE-cadherin mRNA, are significantly elevated in infiltrating breast carcinoma [27] and haematological malignancies [32], respectively.

Of note, the presence of CEPCs correlates with angiogenic activity during tumour growth, and anti-angiogenic therapies reduce their numbers in patients with lymphoma [33], multiple myeloma [34] and acute myeloid leukaemia [35]. In contrast, patients who do not or only partially respond to first-line therapy tend to have increased or stable CEPCs levels. These data are in agreement with several preclinical studies demonstrating the
importance of measuring CEPCs as a surrogate marker of sensitivity to anti-angiogenic and chemotherapeutic agents [4].

Mechanisms of Tumour Vasculogenesis: Multi-Step and Multi-Factor Events

Neoangiogenesis involves EPCs as well as ECs co-opted from surrounding vessels. The mobilisation, recruitment, homing and incorporation of EPCs into tumours are multi-step and multi-factor events. This complex process requires the participation of multiple factors, including growth factors, tumour cells, ECs, stromal cells and EPCs in the tumour microenvironment (fig. 1).

An increase in tumour mass during tumour growth leads to a hypoxic environment, which results in the production of pro-angiogenic growth factors and the onset of the angiogenesis switch [36]. The pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor-BB (PDGF-BB), are involved in the activation, mobilisation and recruitment of BM-EPCs, and in promoting the differentiation of EPCs into ECs in some ischaemic diseases and during tumour growth [37]. Furthermore, these factors activate matrix metalloproteinases (MMPs), particularly MMP-9, leading to the release of soluble KIT ligand, which in turn promotes cell proliferation and migration within the BM microenvironment [38].
Apart from pro-angiogenic factors, stromal cells and EPCs themselves play key roles in the EPC dynamics during tumour vasculogenesis. Spring et al. [22] provided evidence that tumour-associated endothelium recruits and leads to adherence of EPCs through chemokines and their receptors in CEPCs. Using a co-implantation tumour xenograft model, it was demonstrated that carcinoma-associated fibroblasts obtained from human breast carcinomas promoted the growth of admixed breast carcinoma cells significantly more than did normal mammary fibroblasts derived from the same patients [39]. Carcinoma-associated fibroblasts promote angiogenesis by recruiting EPCs into carcinomas, an effect mediated in part by stromal-cell-derived factor-1.

Controversies regarding the Contribution of EPCs to Tumour Vasculogenesis

The existence of EPCs and their selective involvement in neovascularisation has attracted considerable interest because these cells may represent a novel target for therapeutic intervention. Before initiating target treatment with EPCs for cancer, there are some key questions to address, in particular the distribution, contribution, origin and differentiation of EPCs in tumours.

The Distribution of EPCs in Tumour Tissues

The distribution of EPCs, with regard to their recruitment and homing, is affected by hypoxia, angiogenic factors and cell adhesion molecules [40]. Vajkoczy et al. [41] investigated the mechanisms of homing and incorporation of EPCs during new blood vessel formation in a tumour model using mouse embryonic EPCs. They showed that EPCs adhered preferentially near the tumour periphery, coincident with the subsequent highest vascular density. Both vascular cell adhesion molecule-1 and cellular fibronectin are expressed at the tumour periphery, the location to which CD34+ cells home. As the tumour grows, EPCs localise near the periphery, reflecting the greater opportunity for adhesion in this region owing to increased angiogenic activity and higher vascular density [42]. Interestingly, in livers with HCC, there were more EPCs in the tissue adjacent to tumours [3]. In this regard, the particular pathology in HCC with liver cirrhosis may be important. Our studies showed that the recruitment and homing of EPCs into tissue adjacent to tumours may be affected by over-expression of HIF-1α, pro-angiogenic factors and cell matrix adhesion molecules resulting from both liver cirrhosis and HCC [3]. The exact mechanism of EPC recruitment and homing into tumour tissues is worthy of further investigations.

The Contribution of EPC during Tumour Vasculogenesis

EPCs have been identified in the circulation and in the vicinity of malignant cells in some tumours. However, their identity and relative contribution to neovascularure formation has remained controversial. Based on transplantation studies in mice using genetically marked donor BM cells transferred to syngeneic, lethally irradiated recipients, the levels of genetically marked integrated marrow-derived ECs in newly formed blood vessels have been reported to be as high as 50% [43], while other studies show somewhat lower, but nevertheless substantial levels, e.g. 10–20% [44]; in contrast, other studies reported much lower levels, e.g. 5% to essentially non-detectable [45]. With respect to clinical studies, Peters et al. [46] analyzed tumours from 6 patients who developed cancers after BM transplantation with donor cells derived from individuals of the opposite sex. FISH studies with gender-chromosome-specific probes in conjunction with fluorescent antibody staining indicated that marrow-derived donor cells contributed to tumour endothelium, but at low levels (about 5% of the total cells on average).

Such conflicting reports can be ascribed to the following three factors. (1) Angiogenesis and vasculogenesis are controlled in balancing nets. The contribution of EPCs in tumour vasculogenesis keeps varying with tumour growth rate and the degree of tumour ischaemia and vascular injury. Different animal models in different investigations induce the variance. (2) The contribution of CEPCs to cancer vessels might depend on the cancer type, grade, organ site and the mouse strain. In a recent study by Duda et al. [47], the frequency of CEPC-derived vessels was 58% in a mammary model of brain carcinoma metastases, whereas this figure dropped to 1.5% in mammary fat pad breast cancers. (3) More definitive methods are required to distinguish vessel-incorporated BM-EPCs and intimately associated perivascular cells.
The Origin of EPCs in Postnatal Vasculogenesis

Most organs harbour resident stem cells as a means to preserve their physiological integrity [48]. The following investigations have demonstrated that the arterial wall also contains resident progenitor cells in a basal state, which could participate in physiological tissue renewal and play a crucial role during vascular growth and remodelling. Yamashita et al. [49] had previously identified Flk-1+ common vascular precursor cells, which formed tubular structures arising from spheroids in vitro and differentiated into both ECs and smooth muscle cells. These structures strikingly resembled those generated by 'side population' progenitor cells from the normal arterial wall of adult mice [50]. Our results showed that EPCs resided in the media and intima of human umbilical veins and these AC133+ cells gradually differentiated into mature ECs in vitro [51]. These findings suggest the existence of a 'vasculogenic zone' in the wall of large and medium-sized blood vessels, which may serve as a stem cell source of vascular cells in forming new blood vessels (fig. 2) [13]. These cells, identified as CD34+ CD31– Flk-1+ Tie-2+, seem to be localised between the smooth muscle and the adventitial layer of the vascular wall, can generate fully differentiated ECs and can be recruited by cancer cells [13]. Apart from having a biological role in vessel walls, ECs with genetic aberrations like tumours may also participate in tumour angiogenesis, particularly in many haematological neoplasms. ECs with such genetic aberrations resemble stem cells to some extent [52]. These findings may be explained by cell fusion [53], uptake of apoptotic bodies from tumour cells [54], or a common origin of tumour cells and ECs from a multipotent haemangioblastic precursor cell [55]. The mechanisms by which ECs acquire the specific genetic alterations of the tumour cells or stem cells remain to be elucidated.

Dogma Challenged: EPCs into Tumour Tissues, Not to ECs, but to Pericytes

Although the main body of the published data until now suggests the generation of mature ECs from both circulating and BM-EPCs, there are also a few reports...
demonstrating in experimental models that CEPCs do not incorporate into tumour vessels [56]. Using multi-channel laser scanning confocal microscopy of whole-mount tissues, Rajantie [57] showed that BM-EPCs do not significantly contribute to tumour-induced neovascularisation. Instead, BM-derived cells serve as the basis for the generation of peri-ECs, e.g. pericytes rather than ECs at sites of tumour neovascularisation [6]. These notions were further validated using haematopoietic chimeras stably expressing GFP in BM-derived tissues [58]. Maybe, most of the putative BM-EPCs that have been described earlier in publications in fact are BM-derived pericycle-like cells and/or peri-endothelial haematopoietic cells which have been misinterpreted as ECs due to their proximity to the vascular lumen. Possibly, the differences in tissues, disease models and ischaemia intensity may be the explanations for this controversy.

EPCs: Targeting Vectors for Tumour Therapy and Diagnosis

EPCs have an in vivo homing specificity for angiogenic sites and are thus potential vehicles for site-specific gene therapy and diagnosis. The new therapeutic approaches have led to tumour targeting by controlling angiogenesis. Davidoff et al. [42] have tested this approach by modifying murine BM-derived cells with a gene encoding an angiogenesis inhibitor, a truncated, soluble form of Flk-1 (sfFlk-1). After transplantation with tsFlk1-expressing BM cells, tumour growth in mice was significantly inhibited compared with tumour growth in control-transplanted mice. In addition, BM transplantation of Tie-2+ haematopoietic stem cells [4] or endothelial progenitor-like cells [59], which were engineered to blunt angiogenesis, slowed the growth of post-transplant tumour challenges. These results suggest that long-term expression of a functional angiogenesis inhibitor can be generated through gene-modified, BM-derived stem cells, and that this approach can have significant anticancer efficacy and systemic anti-tumour responses without toxicity. However, therapeutic targeting with EPCs will predominantly affect late-stage tumours and may require combination with other, earlier-acting modalities. Thymidine-kinase-expressing EPCs, combined with ganciclovir treatment, induced significant tumour necrosis in animals, with no systemic toxicity [59]. Apart from being target-specific vectors for targeting therapy, EPCs, labelled with ferumoxide-protamine sulphate and observed by positron emission tomography or magnetic resonance imaging, accumulated in the tumour tissue in a time-dependent manner [60]. In summary, a combination of biological modifiers, gene therapy and cell therapy would hopefully provide an efficient means to combat neoplasms, but further studies are needed to realise such an aim.

Future Perspectives

EPCs have been identified in vasculogenesis during tumour progression. Moreover, the characterisation of tumour-associated EPCs may provide valuable new clues for more specific anti-angiogenesis therapy and/or tumour diagnosis in vivo. However, there is a great debate about the nature of mechanisms of EPC mobilisation, migration, differentiation, distribution and homing to the target areas. Therefore, the characterisation of EPCs, growth factors and cells related to EPC mobilisation and differentiation are worthy of further investigations.

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References


