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Involvement of endothelial progenitor cells in tumor vascularization

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ABSTRACT

The generation of new microvasculature progresses by the process of angiogenesis, which involves the invasion and proliferation of endothelial cells from existing blood vessels into the local environment. In recent years, *de novo* generation of endothelial cells from circulating or local precursor endothelial cells is thought to also contribute to the neovasculature, a process that is referred to as vasculogenesis. In the adult, endothelial progenitor cells (EPC) are believed to be recruited from the bone marrow, migrate to sites requiring neovascularization and participate in the assembly of newly-forming blood vessels. A growing number of studies report that EPC participate in tumor progression and influence the efficacy of anticancer chemotherapeutics, and thus are attractive targets for cancer treatments. However, recent evidence calls into question the ability of marrow-derived EPC to act as a *bona fide* precursor for adult vasculogenesis. This review focuses on studies reporting or precluding the importance of EPC in tumor vasculogenesis. The putative sources of these cells and difficulties associated with their detection are discussed.

Introduction

A key step for tumor growth and metastasis is the establishment of new blood vasculature. Until recently, it was thought that neovascularisation, the process by which new blood vessels form, only occurred via angiogenesis in the adult. Angiogenesis is the sprouting of new blood vessels from already-established vessels; this is in contrast to vasculogenesis, which refers to the *de novo* formation of blood vessels from endothelial progenitors, and was thought to occur only in the embryo. Endothelial progenitor cells (EPCs) are classically defined as precursor cells recruited from the bone marrow (BM) that incorporate into nascent vessels at angiogenic sites, proliferate and differentiate into the endothelial cells (EC) that constitute the blood vessel. It is important to mention that some authors refer to BM-derived cells recruited to the periphery of early progressing primary tumors and micrometastatic lesions, where they contribute to the angiogenic switch via the paracrine secretion of pro-angiogenic cytokines, as EPC (Gao and Mittal, 2009). These cells are of myeloid derivation and do not differentiate into mature EC. In the present manuscript, the definition of EPC will be restricted to progenitor EC that structurally integrate into the nascent vasculature and differentiate into mature EC. EPC are distinct from circulating endothelial cells (CEC), which refers generally to a mature population of EC derived from blood vessel turnover that has limited proliferation capacity compared to EPC (Rafii and Lyden, 2003).

Since first reported by Asahara et al. (1997), the role of EPC in tumor progression has raised considerable attention. Studies suggest that EPC facilitate the initial establishment of tumor endothelium (Nolan et al., 2007), control tumor growth (Lyden et al., 2001) and metastasis transition (Gao et al., 2008), and can also determine the sensitivity of a tumor to chemotherapeutics (Shaked et al., 2006; Shaked et al., 2008). Moreover, clinical studies report that the presence of circulating EPC positively correlates with advanced invasive stages, and decline of EPC numbers correlates with response to chemotherapy (Igreja et al., 2007; Naik et al., 2008). This suggests that EPC numbers could potentially be a surrogate marker of both cancer progression and chemotherapeutic efficacy. Other studies support EPC as a potential target for chemotherapy (Shaked et al., 2006, 2008).

Despite the enthusiasm that EPC have raised in recent years, an increasing number of studies suggest that integration of BM-derived EPCs is rare in tumor vasculature, and thus their role as direct precursor cells for tumor EC is questionable. This review focuses on studies related to putative tumor EPCs that are reported to contribute (or not) directly to tumor neovessels by integrating into the vessel wall and differentiating into mature EC.

Potential sources of EPC

Hematopoietic stem cells (HSC) have been observed to arise via budding from the ventral aspect of the embryonic dorsal aorta in the

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aorta-gonad–mesonephros region, suggesting endothelial derivation (Mukouyama et al., 1998; Smith and Glomski, 1982; Tamura et al., 2002; Tavian et al., 1996). Recent advances in mouse genetics, using endothelial lineage tracing systems (e.g., VE-cadherin-CRE x Floxed ROSA-YFP) has shown that these HSCs are indeed produced from aortal endothelial cells (Zovein et al., 2008). These data suggest that, rather than both definitive HSCs and ECs coming from a common precursor (the hemangioblast), arterial hemogenic endothelium is the true precursor to adult blood (Li et al., 2005). After they arise embryonically, HSCs migrate to the fetal liver where multilineage hematopoiesis occurs. Thus, the fetal liver becomes the reservoir of HSC, which can give rise to all hematopoietic lineages necessary during the remainder of development. In post-natal life the HSC pool moves to the BM. Thus, the relationship between EC and blood cells is even more interconnected than previously appreciated.

Previous studies suggested that EPC can be isolated from adult sources such as peripheral blood and bone marrow (Asahara et al., 1997; Peichev et al., 2000; Shi et al., 1998). At least three potential cell types, which are BM-derived, have been suggested to represent the precursor to the adult EPC: HSC and/or hemangioblasts, myeloid cells, and marrow-derived mesenchymal stem cells (Fig. 1).

Hemangioblast/hematopoietic stem cells

Studies have reported that EPC that fulfill the previous description of the embryonic hemangioblast can be isolated in the adult from BM or blood. The first study identifying adult EPCs showed that $CD34^+$ cells isolated from circulating mononuclear blood cells displayed molecular and phenotypic changes associated with mature EC under the appropriate conditions (Asahara et al., 1997). Circulating EPC are thought to be included in the cell population expressing CD133 and VEGFR-2 within the subset of $CD34^+$ cells (Gill et al., 2001) but several other markers are used to identify EPC, which vary depending on the studies (Table 1). The most stringent criteria to define EPCs in mouse are Sca-1^+/Kit-1^+/Lin$^-$/VEGFR-1^+; while in human $CD34^+$/c-kit ^{low}/Lin$^-$/$CD133^+$/VEGFR-1^+/VEGFR-2^+ are used as putative EPC markers (Larrivee and Karsan, 2007). Many, if not most, of these markers are also used to define hematopoietic cell populations as discussed below and shown in Table 2, which hampers the identification of a cell as truly being an EPC. CD133 is commonly used to distinguish EPC from CEC and myelomonocytic cells, as this stem cell associated marker is not expressed by CEC and is lost from the surface of myelomonocytic cells (Rehman et al., 2003). However, the utility of CD133 expression in mouse as a specific stem-cell marker is not clear, and some studies rely on CD117 as a surrogate marker of CD133 in mouse (Bertolini et al., 2006).

Circulating human EPC defined by $CD34^+$/$VEGFR2^+$ lose CD133 expression upon differentiation into mature EC. HSC also lose CD133 expression as they commit to a differentiated progenitor stage (Peichev et al., 2000). In the presence of VEGF, cultures of primitive $CD133^+$ hematopoietic progenitors give rise to colonies (CFU-EC, as

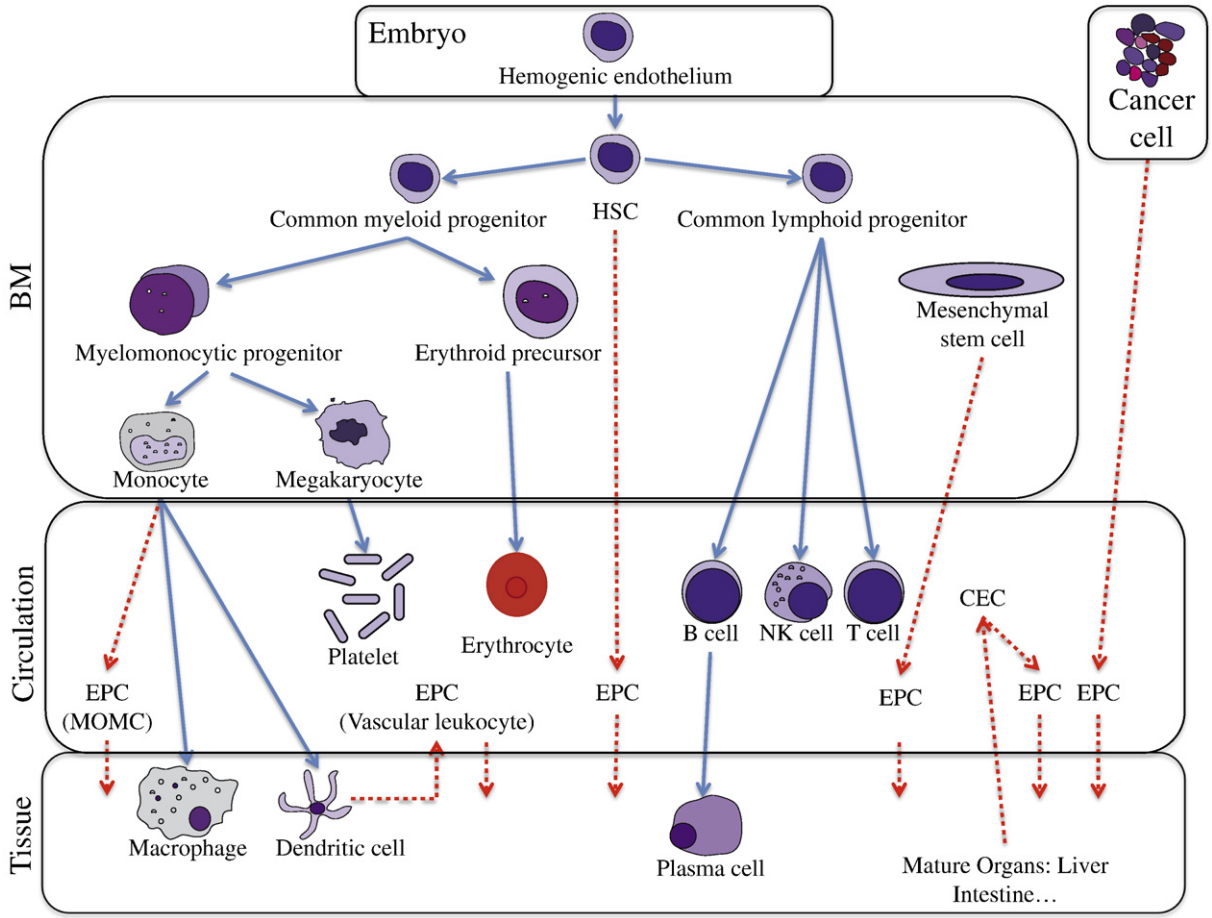

Fig. 1. Putative sources of EPC. Hemogenic endothelium gives rise to HSC during embryonic development. At the adult stage, BM is responsible for the maintenance of the HSC pool, and gives rise to cells which undergo differentiation into myeloid and lymphoid cell lineages. EPC have been suggested to arise from HSC or progeny cells such as monocytes and dendritic cells referred to as monocyte-derived multipotential progenitor cells (MOMCs) and vascular leukocytes, respectively. Mesenchymal stem cells (MSC) are recruited to the tumors and can differentiate into EC. Circulating endothelial cells (CEC) from organs such as liver and intestine are also suggested to be a potential source of EPC. Cancer stem cells have been suggested to differentiate into blood vessel structures that express EC molecular characteristics in tumors.