Microvascular Research 79 (2010) 224–228

Contents lists available at ScienceDirect

Microvascular Research

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

Circulating endothelial cells as biomarkers in clinical oncology

Patrizia Mancuso, Francesco Bertolini *

Laboratory of Hematology-Oncology, European Institute of Oncology, Milan, Italy

ARTICLE INFO

Article history:
Received 25 November 2009
Accepted 11 February 2010
Available online 20 February 2010

Keywords:
Angiogenesis
Endothelial cells
Endothelial progenitors

ABSTRACT

Circulating endothelial cells (CECs) and circulating endothelial progenitors (CEPs) play a different role in cancer development, acting as possible markers of vascular turnover/damage (CECs) and vasculogenesis (CEPs). Preclinical and clinical data suggest that CEC enumeration might be useful to define the best treatment option for patients who are candidate to anti-angiogenic therapy, while CEPs seem to have a "catalytic" role in different steps of cancer progression and recurrence after therapy. The definition of CEC and CEP phenotype and the standardization of CEC and CEP enumeration procedures are highly warranted to use these cells as biomarkers in clinical trials in oncology, and to compare results from different studies.

Contents

Introduction . . . 224
CEP and CEC phenotype . . . 224
CEC number and viability in cancer and treatment . . . 225
CEPs in tumor grow and metastases . . . 226
CEC and CEP as tools to monitor minimal residual disease . . . 226
CEC/CEP in the clinic . . . 227
Conclusions . . . 227
Acknowledgments . . . 227
References . . . 227

Introduction

The endothelial cell turnover has always been thought to be very slow compared with other tissues. This notwithstanding, cells with endothelial morphology were found to circulate in the blood more than 35 years ago (Hladovec and Rossamn, 1973). In the following years, the endothelial nature of these cells was confirmed by immunohistochemistry (IHC) studies, and their enumeration by means of positive enrichment, IHC or flow cytometry (FC) indicated that circulating endothelial cells (CECs) are increased in a very wide spectrum of disorders encompassing vascular, autoimmune, infectious and ischemic diseases (Moldovan et al., 1994; Bertolini et al., 2006). Over the past 10 years, increased CEC counts were observed in some cancer patients (Mancuso et al., 2001; Farace et al., 2007), and these cells were studied as surrogate biomarkers of angiogenesis and anti-angiogenic drug activity in preclinical models and medical oncology (Monestiroli et al., 2001; Shaked et al., 2005a,b; Calleri et al., 2009). These studies also indicated that the endothelial phenotype was expressed by cells displaying a wide variety of different features (Blann et al., 2005; Bertolini et al., 2006). Some CECs had a phenotype compatible with terminally differentiated endothelial cells (EC), in some cases being apoptotic or necrotic and thus most likely derived from the turnover of vessel walls. Some other cells expressed progenitor-associated antigens in addition to endothelial antigens, and were considered as circulating endothelial progenitor (CEP) candidates.

CEP and CEC phenotype

There is a lack of consensus regarding the surface markers that identify CEPs and CECs, due to the lack of a marker that can unambiguously identify these cells.

* Corresponding author. Laboratory of Hematology-Oncology, Departments of Pathology and Medicine, European Institute of Oncology; via Ripamonti 435, 20141 Milan, Italy. Fax: +39 02 57489537.
E-mail address: francesco.bertolini@ieo.it (F. Bertolini).

0026-2862/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.mvr.2010.02.007

By means of flow cytometry, the most widely used antigens to identify CEPs are CD34, VEGFR2 and CD133. Many investigators identify and enumerate CEPs by means of the co-expression of CD34 and VEGFR2 (Rosenzweig, 2005; Bertolini et al., 2006; Asahara et al., 1997; Shi et al., 1998), but these antigens are expressed also by mature CECs. The sole antigen that according to some authors is expressed on progenitors but not on mature endothelial cells is CD133. Unfortunately, in humans CD133 is expressed also by hematopoietic stem cells (HSCs), and other progenitors from neural and gastro-intestinal tissues (Mizrak et al., 2008). Moreover, the use of CD133 for CEP identification (Peichev et al., 2000; Rafii et al., 2002) has led to the isolation of cells that not all laboratories were able to differentiate in vitro and in vivo along the endothelial lineage (Yoder et al., 2007; Case et al., 2007). More specific markers are thus needed to distinguish CEPs from CECs and HSCs.

Even more controversies exist for the enumeration of CEP in mice, because the expression and function of CD34 and CD133 antigens are less well characterized in mice than in humans. A candidate CEP phenotype in mice is CD45−, VEGFR2(Flk)+, CD117+ (Monestiroli et al., 2001; Shaked et al., 2005a,b), and antibodies reacting with a particular configuration of CD144 are also used (Nolan et al., 2007; Gao et al., 2008). This controversy regarding CEP phenotype in mice has led in the past to opposing conclusions regarding CEP contribution to cancer vasculogenesis, with some studies indicating a crucial role for CEPs and some others failing to observe a relevant CEP contribution (reviewed in Madlambayan et al., 2009).

Antigenic promiscuity between CECs and platelets has prompted the development and validation of new FC enumeration procedures were DNA staining reagents have allowed the enumeration and isolation of platelet-depleted, DNA-containing cells with an EC phenotype (CD45−,CD31+CD146+) (Mancuso et al., 2009). Transmission electron microscopy (TEM) and RT-PCR of sorted CECs confirmed their endothelial nature by virtue of the presence of EC-specific Weibel–Palade bodies and of RNA transcripts for the EC-specific gene VE-cadherin. TEM studies also offered an explanation of the controversies about CEC frequency in the blood. The majority of sorted CECs, in fact, were found to be apoptotic or necrotic cellular fragments, most likely lost at count after the cell processing involved in IHC enumeration. Along with apoptotic CECs, however, TEM showed the presence of small, viable and lymphoid-like cells that are compatible with a progenitor cell morphology (Fig. 1).

TEM will most likely be of help for the next crucial steps in CEC and CEP studies, i.e. to dissect the functions of candidate CEC and CEP subpopulations. Both these cell families, in fact, encompass subpopulations with different roles. Multiparametric FC has shown that among DNA+,CD45−,CD31+,CD146+ CECs there are some expressing other EC-related antigens such as CD143, CD144, VEGFR1, VEGFR2, VEGFR3, activation antigens such as CD105 (endoglin), and others (Bertolini et al., 2006).

CEC number and viability in cancer and treatment

Endothelial cell enumeration (both CECs and CEPs) has led to the observation that these cells are increased and are more viable in some types of cancer patients compared to healthy controls (Mancuso et al., 2001; Farace et al., 2007) (Fig. 2). In breast cancer, CECs and CEPs demonstrated a strong relationship with the Nottingham prognostic index (NPI), but only CECs positively predicted higher NPI scores and correlated with tumor invasiveness and size, possibly reflecting total tumor vascular volume (Goon et al., 2009). Possible explanations for these findings involve the angiogenic switch associated with cancer growth and the robust production of angiogenic growth factors such as VEGF, bFGF, HGF and many others by cancer cells and/or various host cells (Bertolini et al., 2006). The recent and unexpected finding of an autocrine loop in EC (Lee et al., 2007) is of particular interest, because it might be that the increase of viable CECs in the blood of cancer patients mirrors an aberrant vascular turnover/remodeling associated with high local levels of VEGF produced by cancer cells.

Following the preclinical evidence that CEC count can be used as a surrogate marker of angiogenesis and of anti-angiogenic drug activity and to define the optimal biological dosage of anti-angiogenic drugs (Shaked et al., 2005a,b), CEC number and viability have been measured in different clinical trials where cancer patients have been treated with anti-angiogenic therapies (Rabascio et al., 2004; Farace et al., 2007). An increase in the number of apoptotic CECs after 60 days of therapy was associated with a prolonged progression-free survival and overall survival in metastatic breast cancer patients treated with a doublet metronomic chemotherapy regimen (Mancuso et al., 2006). When the anti-VEGF antibody bevacizumab was added to metronomic chemotherapy for the therapy of metastatic breast cancer, patients who had a clinical response (as well as the larger population of patients who had a clinical benefit from the treatment) had significantly greater baseline levels of viable CECs than patients who did not respond to therapy. Also, the number of apoptotic CECs before the beginning of therapy was associated with a prolonged

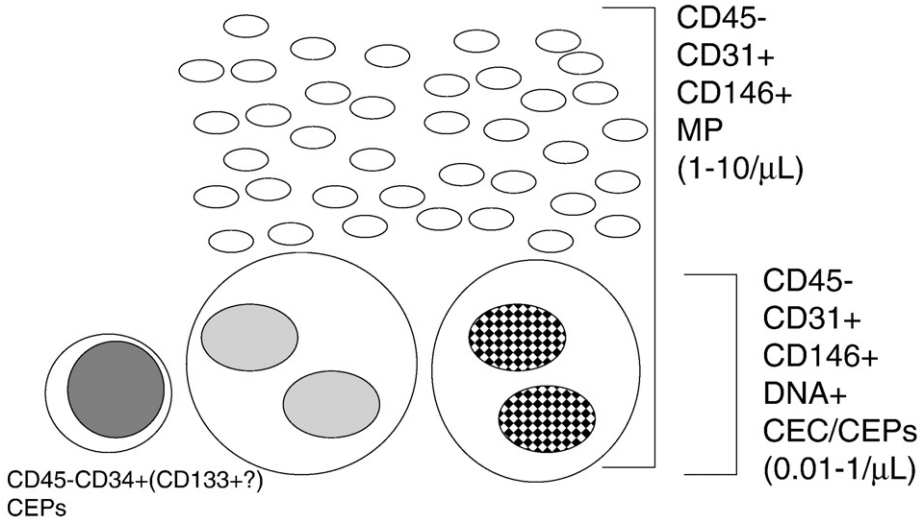

Fig. 1. Rendering of the different cellular events with an endothelial phenotype enumerated by flow cytometry, including CD45−,CD31+CD146+ macroparticles from apoptotic or necrotic CEC fragments, DNA-containing CD45−CD31+CD146+ viable or apoptotic CECs, and lymphoid-like CEPs expressing CD133 and/or CD34 progenitor markers (Mancuso et al., 2009).