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Original Research

Breast and renal cancer—Derived endothelial colony forming cells share a common gene signature

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KEYWORDS
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Abstract ***Background:*** Neovascularisation supports the metastatic switch in many aggressive solid cancers. Tumour neovessels are mostly lined by endothelial cells sprouting from nearby capillaries, but they could also be contributed by circulating endothelial progenitor cells (EPCs). However, scant information is available about tumour-derived EPCs.

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Endothelial progenitor cells;
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Gene profiling

Methods: We carried out the first thorough, unbiased comparison of phenotype, function and genotype of normal versus tumour-derived endothelial colony forming cells (ECFCs), a truly endothelial EPC subtype. We used healthy donors–derived ECFCs (N-ECFCs) as control for breast cancer (BC)– and renal cell carcinoma (RCC)–derived ECFCs.
Results: We found that both BC- and RCC-ECFCs belong to the endothelial lineage. Normal and tumour-derived ECFCs did not differ in terms of proliferative and tubulogenic rates. However, RCC-ECFCs were more resistant to rapamycin-induced apoptosis, whereas BC-ECFCs were more sensitive as compared with N-ECFCs. Gene expression profiling revealed 382 differentially expressed genes (DEGs; 192 upregulated and 150 downregulated) and 71 DEGs (33 upregulated, 38 downregulated) when comparing, respectively, BC- and RCC-ECFCs with N-ECFCs. Nonetheless, BC- and RCC-derived ECFCs shared 35 DEGs, 10 of which were validated by qRT-PCR; such 35 DEGs are organised in a gene network centred on FOS.
Conclusion: These results provide the first clear-cut evidence that BC- and RCC-derived ECFCs exhibit an altered gene expression profile as compared with N-ECFCs; yet, they share a common gene signature that could highlight novel and more specific targets to suppress tumour vascularisation.

1. Introduction

Tumour vascularisation supports tumour growth and metastasis and serves as a target for anti-angiogenic drugs in cancer patients [1,2]. Tumour endothelial cells (TECs) display dramatic genetic, cytogenetic, epigenetic and morphological aberrations as compared with their healthy counterparts [3]. The alterations in TEC phenotype may be dictated either by tumour microenvironment (TME), which releases soluble mediators and exosomes to reprogram local endothelial cells [4,5], or by the original site-specific epigenetic footprint [6]. An additional source of heterogeneity to TECs is represented by the intrinsic dynamics of tumour vascularisation, which impinges on mechanisms other than sprouting angiogenesis, such as the recruitment of bone marrow (BM)–derived endothelial progenitor cells (EPCs), according to a process termed vasculogenesis [1,3]. Albeit highly debated [2], EPCs might sustain the angiogenic switch through the paracrine release of further growth factors or by physically engrafting within neovessels [2,7,8].

Endothelial colony forming cells (ECFCs) represent the only EPC population truly belonging to the endothelial lineage [9] and capable of forming capillary structures *in vitro* and patent vessels *in vivo* [10]. ECFCs show an innate tumour tropism and sustain the angiogenic switch in many solid cancers [11–13]. ECFCs do not belong to the neoplastic clone [14] and, in principle, should not be affected by TME. Nevertheless, ECFCs isolated from tumour patients exhibit a cancer-specific rearrangement of their Ca^{2+} signalling machinery [15–17] which is central to ECFC proliferation and tubulogenesis [18]. Thus, neoplastic transformation could exert significant modifications on circulating ECFCs. There is, therefore, the urgent need to conduct a more insightful evaluation of the gene expression profile and a functional module analysis to understand whether and how ECFCs isolated from different cancer types differ from each other and from normal cells. This would be extremely useful to get a deeper insight into the molecular and cellular mechanisms of tumour vascularisation.

The present study was undertaken to obtain a thorough, unbiased phenotype and genotype comparison of normal versus tumour-derived ECFCs. We focussed on two totally distinct solid cancers, such as renal cell carcinoma (RCC) and breast cancer (BC), which yet share the need of robust angiogenesis and require EPCs for their growth and metastasis [19,20].

2. Patients and methods

Blood samples (40 mL) were obtained from healthy human volunteers aged from 22 to 48 years old (n = 34), from BC patients (n = 68) and from RCC patients (n = 37). Demographic and clinical characteristics of patients are summarised in Supplementary Table 1 and Supplementary Table 2 for BC and RCC, respectively. The Institutional Review Boards at 'Istituto di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo Foundation' and at 'Istituto di Ricovero e Cura a Carattere Scientifico Fondazione Salvatore Maugeri' in Pavia approved all protocols for the study. Informed written consent was obtained according to the Declaration of Helsinki.

ECFCs isolated from peripheral blood were evaluated in terms of functional differences as described in Supplementary Materials section. Gene expression profiles and signature validation were as described in Supplementary Materials section.