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Current Perspective

From cancer patients to cancer survivors: The issue of Cardioncology – A biological perspective

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ABSTRACT

Long-term survival of cancer patients can be worsened by cardiovascular morbidity and mortality due to anticancer treatments based on cardiotoxic or antiangiogenic regimens. Growing scientific evidences support a role for circulating endothelial progenitor cells (EPCs) both in cancer pathogenesis and in cardiovascular diseases. High frequency of circulating EPCs seems to play a role in cancer growth and dissemination by favouring tumor angiogenesis and estabilishment of sites of metastasis. On the other hand, high level of circulating EPCs seems to be associated with a lower risk of developing cardiovascular diseases and with improved vascular regeneration after cardiovascular damage. Here, the possibile opposing roles of circulating EPCs in cancer patients suffering from therapy related-cardiovascular diseases are discussed, under the light of the potential modulation of their levels for therapeutic purposes. This can become a relevant issue in the field of cardioncology, the discipline that deals with the managing and treatment of cancer patients suffering from concomitant cardiovascular diseases or who are exposed to an increased risk to develop therapy related-cardiovascular complications.

1. Cardioncology: the need for a renewal partnership between oncologists and cardiologists

With the crude incidence estimated at 3.2 million new cases each year, today in Europe a person is diagnosed with cancer every 10 s, and the ageing of the population will cause this number to increase continually in the future.

Substantial improvements in cancer therapy and survival have been attained in recent years. According to the EUROCARE-4 study results, there are approximately 16 million people still alive in Europe (corresponding to 2–3% of the entire European population) who had a diagnosis of cancer in the last decade.[1] As the number of cancer survivors increases and their length of survival extends, long-term health issues specific to this population are fast emerging as a public health concern.

The impact of cancer and cancer treatment on the long-term health of these survivors is particularly relevant in the case of cardiovascular diseases. Robust evidence about cardiac risk associated to anticancer treatment was provided through childhood cancer survival follow-up. The Cancer Survivors Study showed that cancer survivors had an 8-fold increased risk of cardiovascular mortality compared to the healthy population 25 years after therapy.[1]

On the other hand, the evaluation of the impact of cardiovascular diseases in adult and elderly cancer patients is made complex by many critical issues. So far, elderly cancer patients

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are underrepresented in clinical trials. Moreover, a significant proportion of adult patients at the time of cancer diagnosis is additionally affected by such cardiovascular comorbidities, which could be significantly worsened by cancer treatment.[2]

Several observations raise concerns that adult-onset cancer survivors might be plagued by increased cardiovascular morbidity similar to that of long-term survivors of childhood cancer. If we consider that up to 300,000 patients with a new breast cancer diagnosis are expected each year in Europe, and that approximately one-third of them will receive adjuvant chemotherapy with an anthracycline-containing regimen and that one in four women over-expresses the HER2-receptor and is therefore treated with trastuzumab, then more than 25,000 breast cancer patients each year in Europe will be at an even greater risk for cardiotoxic complications during or after treatment.[3]

In this scenario, the improvement in cancer quality and quantity of life could potentially vanish because of the cardiovascular morbidity and mortality associated with anticancer treatment. The emerging need to treat cancer whilst sparing the heart is reminiscent of Ulysses' navigation of the strait of Messina, where he steered away from the whirlpool Charybdis, only to fall into the jaws of the sea monster Scylla.

This issue calls for a renewal partnership between oncologists and cardiologists, and the common field of challenge is now 'Cardioncology'. Cardioncology is defined as a new interdisciplinary area of research and treatment focused on the growing category of cancer patients affected by concomitant cardiovascular disease and of cancer patients whose treatments expose them to the risk of cardiovascular complications.

The first important issue of Cardioncology should be to promote investigations to clarify the epidemiological magnitude of cardiovascular complications in cancer patients, and to identify individual risk factors of developing cardiovascular toxicity. In addition, there is a need to identify early signs of cardiac damage in order to optimise the clinical management of cardiotoxicity. Finally, tailored strategies aimed to balance the risk of cardiotoxicity with clinical benefit are warranted.

Cardiovascular complications resulting from conventional anticancer treatments are very heterogeneous. In addition, with the introduction of new classes of drugs and in particular of tyrosine kinase inhibitors (TKIs), both in the form of monoclonal antibodies (trastuzumab, bevacizumab) or small molecules (sunitinib, sorafenib lapatinib), it is becoming clear that these compounds might also have significant side effects involving the cardiovascular system.[4]

The extreme variety of clinical phenotypes of cardiovascular complications in cancer patients underlines the importance to clarify the primary mechanisms of cardiotoxicity of anticancer drugs and to understand the possible interactions in combination therapies. In this context, the study of endothelial cell compartment is becoming increasingly important for several reasons.

First, growing scientific evidence supports the concept that endothelial cells are an important target in the physiopathology of cardiovascular damage from both conventional and new anticancer treatments. Myocytes comprise approximately 80% of the cardiac mass but constitute less than 20% of the total cell count. Other cell types, in particular endothelial cells, provide structural and trophic support to the myocytes: the effect of anticancer treatments on these cells needs to be evaluated. In this context, the evidence that oxidative stress is the sole cause of chronic cardiotoxicity by conventional treatments in humans is inconclusive.[5] As far as 'new' drugs (TKIs) are concerned, at present, mechanisms of treatment-related cardiovascular diseases remain largely unknown. TKs play a central role in the activation of signalling pathways that regulate cell growth, differentiation, metabolism, migration and apoptosis. Many of these pathways are implicated in cancer proliferation and tumour angiogenesis, and may also regulate survival of normal cells, including cardiomyocytes and endothelial cells. Targeting TK in cancer cells and angiogenesis may lead to cardiovascular diseases because of inhibition of these same prosurvival signals relevant to the physiological cellular activity in the heart and associated vessels.[4,6]

Second, recent findings have significantly improved our understanding of the angiogenesis process in both physiological and pathological conditions. In 1997, Asahara et al. reported, in a seminal paper published in Science, the identification, in the peripheral blood (PB) of adult, healthy subjects, of a bone marrow (BM)-derived cell with the features of a progenitor and belonging to the endothelial lineage, that he called the endothelial progenitor cell (EPC).[7] The finding that cells capable of giving rise, after *in vitro* proliferation and differentiation, to mature endothelial cells circulated in the peripheral blood was regarded as a crucial contribution to the field of angiogenesis. In fact, the observation that circulating EPCs also exist in adult individuals challenges the paradigm that new vessel formation in post natal life occurs only through a process called neoangiogenesis, in which new vessels sprouts from pre-existing vessels, whereas vasculogenesis, i.e. the formation of new vessels from circulating endothelial precursors, exclusively occurs in the developing embryo and in the foetus.[8]

In the light of Cardioncology, it seems attractive to speculate that circulating EPCs may play a pivotal role both in the tumour angiogenesis and in the vascular regeneration occurring after cardiovascular damage in cancer patients under treatment with antiangiogenic or cardiotoxic therapeutics.

In this paper we discuss methodological issues about the identification of EPCs as well as the contribution of EPCs to both cancer angiogenesis and vascular homeostasis in the cardiovascular system.

2. Endothelial progenitor cell identification

Since their identification, an increasing number of papers focusing on the enumeration, characterisation, and functional role in vascular homeostasis of EPCs have been published, sometimes reporting conflicting results. Paradoxically, despite the large amount of research performed on EPCs, the debate about their definition, identification and functional characterisation (*in vitro* and *in vivo*) is still open and a definitive agreement and standardisation of these issues has not yet been reached.

For the identification of circulating EPCs, two, not mutually exclusive, approaches have been used since they were first described: i) *in vitro* cultures and ii) immunophenotypical analysis by FACS. The latter could be the best method to iden-

tify and enumerate EPCs: it is fast to perform and requires few mL of PB; it is not dependent on biological properties such as the capacity to grow *in vitro* and it can also identify those progenitor cells that are not clonogenic in the actual available assays (see below); once standardised, it is easily reproducible in different laboratories. Unfortunately, surface protein expression turned out to be insufficient to identify EPCs, despite the large amount of studies devoted to this aim, because the most commonly used antigens for EPC detection (such as CD34, CD133, VEGFR-2, CD133 and CXCR4) are also expressed by haematopoietic stem/progenitor cells (HPCs).[9,10] Very recently, however, a new promising cytofluorimetric approach for the identification of circulating mature endothelial cells and EPCs has been proposed, which is based on the detection of viable (Syto16+) CD45-CD31+CD146+ cells.[11] *In vitro* cell culture experiments have identified, up to now, three types of EPCs: the so-called Colony Forming Unit-Endothelial Cells (CFU-End), also abbreviated as CFU-EC (or CFU-Hill, or early EPCs); the Circulating Angiogenic Cells (CACs), also sometimes called early EPCs; and the Endothelial Colony Forming Cells, also known as ECFC (or Blood Outgrow Endothelial Cells, BOECs, or late EPCs). CFU-Ends are the originally EPC-derived colonies described by Asahara and subsequently by others with minor modification of the original methods. They derive from the non adherent fraction of the circulating mononuclear cells and appear early in culture (after 1 week incubation).[7,12] Characterisation of these putative EPCs has shown that they do not possess self-renewal capacity and have a modest proliferation activity:[13] thus, they do not meet the main criteria for being considered real progenitor cells. More importantly, they express a bi-lineage phenotype (both endothelial and haematopoietic) which suggests that they derive from cells of myeloid origin: in fact, studies in patients with myeloproliferative diseases carrying the V617F JAK-2 mutation have shown that CFU-Ends are clonally related to the myeloid malignant cells that support the disease.[13,14] Finally, in animal models they were not able to directly contribute to neovessel generation although a paracrine role in such a process is probably played.[15,16] CACs also derive from the circulating mononuclear fraction of PB cells; however, differently from CFU-End, they stem from the adherent fraction of mononuclear cells.[17] *In vitro*, they also appear within a few days from the beginning of the culture as single, spindle shaped cells that firmly attach to the culture dish: like CFU-Ends, they are not capable of self-renewing and proliferating and display a mixed haematopoietic-endothelial immunophenotype. It was very recently demonstrated that at least part of the endothelial surface proteins expressed by these cells is due to the uptake of platelet microparticles that form *in vitro* in the culture assay in which CACs are grown.[18] *In vivo* administration of CACs displayed an effective contribution to neovasculogenesis in animal models; however, clinical trials in patients with cardiovascular diseases did not end up in any significant clinical improvement.[19,20] The third type of *in vitro* identified EPC is the ECFC which derives from the adherent fraction of circulating mononuclear cells, does not appear in culture before 10–12 days, and displays self-renewal capacity and a great proliferative potential.[21] ECFCs are organised in a hierarchical system (similar to haematopoietic progenitors) and show a faithful belonging to the endothelial lineage by expressing only endothelial and not haematopoietic surface proteins. In JAK-2 positive myeloproliferative patients, they are not clonally related to the haematopoietic malignant clone that sustains the disease[13,14] and, finally, they are able in animal models to directly participate in neovasculogenesis in an effective and efficient way.[13] Thus, ECFC meets all the criteria of a progenitor cell and represents up to now the unique and reliable surrogate of actual, real EPCs; the ECFC assay should be regarded as the only way to identify and measure circulating EPCs. According to the original description,[21] ECFC frequency in steady state PB of adult healthy individuals is very low, ranging from 0.05 to 0.2 cells per mL. However, in a very recent report, Rainesch and colleagues have described an improved culture system that detects a higher frequency of ECFC and allows the start of the culture with just a few mL of PB.[22] If only ECFC can be regarded as "true" EPC what is the role, if any, of early EPCs in neovasculogenesis? It has been suggested by different approaches that early and late EPCs may work in a synergistic way:[23] ECFCs directly participate in the formation of new vessels being incorporated into developing vascular networks[13,24] whereas early EPCs (CFU-Ends and CACs) and, very likely, other BM-derived myeloid cells, participate by secreting paracrine factors which favour and promote neovascularisation by recruiting late EPCs and orchestrating their activity.[16,24–26]

3. EPCs and cancer

Both the growth of a tumour and its process of metastasisation can occur only if new vessel formation is provided.[27] It is therefore not surprising that several studies have been performed on the role played by endothelial cells (both mature and progenitor) in tumour growth and progression. With specific regard to circulating EPCs, compelling evidences suggest that they are involved both in cancerogenesis,[28–30] and in the formation of what has been called by some researchers the pre-metastatic niche.[29,31] Following the formation of the pre-metastatic niche, other EPCs together with tumour cells move to this niche and form the metastasis.[29,31] Moreover, a role in tumour vascularisation has also been suggested for the recently described vessel wall resident EPCs[32] and, to make the tumour-associated angiogenic machinery more complex, it is likely that other cells, namely haematopoietic progenitor cells and mature myeloid cells, co-operate together with circulating EPCs in tumour and metastasis vascularisation.[33,34]

The entity of contribution of EPCs to tumour vasculature is, however, variable and in some reports a negligible role, if any, of circulating EPCs in cancer growth has been described.[35–37] These apparently conflicting results might be explained by the fact that the contribution of EPCs to cancer growth and metastasis can vary, depending on type, stage and location of tumour as well as by methodological experimental issues, such as the animal model, the number and type of transplanted cells, and the markers used for endothelial cell detection. Thus, not only neoangiogenetic processes (as traditionally described[38]) but also neovasculogenesis, provided by circulating EPCs, contribute to tumour and metastasis neovessel formation (for an extensive review on the role of EPCs in cancer angiogenesis see Bertolini and colleagues[39]).

Another issue to be considered is the belonging of EPCs to the malignant clone. In fact, endothelial cells of neoplastic origin can be not infrequently detected in many adult and paediatric tumours (melanoma, multiple myeloma, renal carcinoma, non Hodgkin lymphoma, neuroblastoma). A reasonable explanation for these observations is that tumour cells can differentiate into endothelial mature (although instable) cells, a phenomenon called vascular mimicry.[40] However, a potential alternative but not exclusive explanation could be the existence of 'neoplastic' circulating EPCs. This could be true especially in haematological neoplasias where the malignant transformation could occur in a progenitor cell common to both the haematopoietic and the endothelial lineage, the so-called haemangioblast. In fact, in recent years, evidences have been published of circulating EPCs harbouring the same genetic or molecular defect present in the malignant clone that sustains some haematological disease, such as multiple myeloma, chronic myeloid leukaemia and idiopathic myelofibrosis.[41–43] In these particular cases, however, it is very likely that early EPCs were studied (which are myeloid in origin and therefore necessarily belong to the malignant clone). When late EPCs (which are 'true' EPCs belonging to the endothelial lineage) have been investigated, the molecular defect was never detected.[13,14] Thus, circulating EPCs, as seen by many evidences, are involved in tumour growth and metastasis but do not derive from the neoplastic clone.

4. EPCs in cardiovascular diseases

A huge amount of studies have been performed in the last decade focusing on the relationship between the level of circulating EPCs and cardiovascular diseases or cardiovascular risk, sometimes ending up with conflicting results (reviewed in Pompilio and colleagues[44]). This is mainly due to the different methodological approaches that were used for EPC detection and enumeration and by the fact that these results were often derived from studies based on small patient populations. Two studies, in which EPCs were identified as early EPCs or CACs, showed that in healthy adult subjects an inverse correlation exists between EPC and cardiovascular risk.[12,45] Similarly, studies by Schmidt-Lucke and Werner have shown that reduced levels of circulating EPCs, assessed as CD34+VEGFR2+ circulating cells, are independently related to atherosclerosis progression and in turn to higher occurrence of cardiovascular events and death.[46,47] If there is a certain degree of agreement that a low level of circulating EPCs predicts an increased risk for the occurrence of cardiovascular diseases, a less clear relationship exists between levels of EPCs and coronary artery diseases (CAD). According to some investigators, EPCs (assessed as CD34+VEGFR-2+ cells[47] or early EPCs[48]) are reduced in patients with chronic CAD compared to age-matched healthy subjects whereas others either found no changes between patients with stable angina and healthy subjects[49] (in this study EPCs were assessed as early EPCs and CD34+CD133+VEGFR2+ cells) or found that circulating EPCs, measured as late EPCs in culture, proportionally increase with the severity of CAD.[50] At least five different studies[49,51–54] found, using distinct approaches for EPC detection, that early phases of acute myocardial infarction (AMI) are characterised by significant increase of circulating EPCs and, in two of them, elevated progenitor cell counts were predictors of favourable improvement of ventricular function.[52,53] Besides these promising, although non conclusive, *ex vivo* results, transplantation of EPCs in animal models of acute MI or hind limb ischaemia also resulted in encouraging outcomes. In immunodeficient rats after ligation of the coronary artery, transplantation of human EPCs resulted in the incorporation of cells into sites of myocardial neovascularisation, differentiation into mature endothelial cells, inhibition of fibrosis and recovery of left ventricular function.[55,56] Similar effects were also reported in models of acute MI in animals of a larger size.[57,58] These *in vivo* studies, together with the potential beneficial effect of EPC mobilisation in AMI and the reports showing a 'protective' effect of high levels of EPCs toward cardiovascular diseases, were the basis for the design of clinical trials in which autologous progenitor cells (including putative EPCs) were administered to patients with either AMI, or chronic ischaemic heart diseases or peripheral ischaemia (reviewed in [59] and [60]). These trials, based on a small number of patients, ended up in discordant outcomes and no definitive agreement on the efficacy of such cellular therapies has been reached up to now. Importantly, in those trials in which an effective outcome was reported, no direct evidence of engraftment of transplanted cells was reported. It is likely that only large scale studies based on the administration of a well defined subset of progenitor cells (including real EPCs) will definitively clarify the effectiveness of such therapies. Until then, treatment of cardiovascular diseases by EPC transplantation or by pharmacological modulation of their number should be considered with caution.

5. Conclusion

Participation of EPCs in neovasculogenesis in adult individuals, both in physiological and pathological conditions, has been investigated in recent years. Although there is evidence that other cells (endothelial-like cells of monocytic origin, haematopoietic progenitor cells, mature myeloid cells) coparticipate in adult neovasculogenesis together with vessel wall resident EPCs, it is likely that circulating EPCs represent the major player of this process. However, a strict, non ambiguous characterisation of the phenotype of what we call EPC is mandatory to completely understand the role(s) that these cells play in neovasculogenesis.

In patients with cardiovascular diseases, a normal or increased amount of circulating EPCs seems to be a favourable condition, being associated with a reduction of cardiovascular risk, a better prognosis of chronic CAD and possibly an improved recovery from acute myocardial ischaemia. In clinical oncology, if it is true that EPCs (co-mobilised with HPCs) play a critical role in tumour angiogenesis, then these cells should represent an ideal target for inhibiting tumour growth. Alternatively, since circulating EPCs seem to display an effective capacity to home to tumour vasculature, favouring both local cancer growth and metastasisation, they could offer the unique opportunity to deliver specific drugs to the sites of the neoplasia. Although most clinical applications of EPCs have been aimed at the recovery of ischaemic tissue (where an increase in the number of these cells is desired), inhibition of

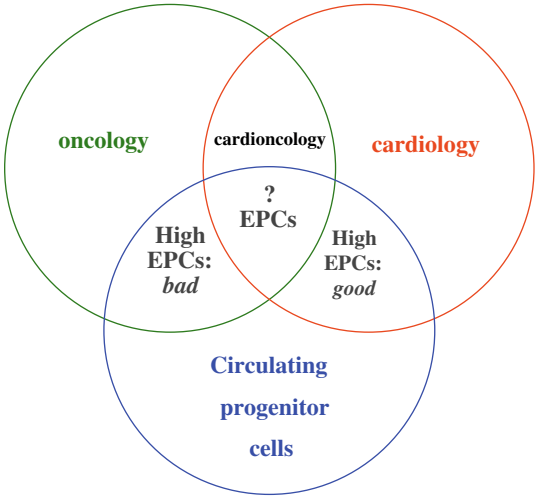

Fig. 1 – A simplification of the role of circulating EPCs in oncology, cardiology, and cardioncology. A high frequency of circulating EPCs is associated with a good prognosis in cardiovascular diseases and could exert a potential beneficial repairing activity. On the contrary, EPCs are involved in tumour growth and progression, as well as metastasisation. Thus, in forthcoming years, reducing circulating EPCs to limit tumour growth or increasing them to limit cardiac damage derived from the use of chemotherapy could become a hotly debated issue in oncology and cardiology.

EPC mobilisation from the bone marrow could have a prominent role in future cancer treatment.

If the large body of preliminary observations on the role of EPCs both in cardiovascular diseases and in cancer pathogenesis are confirmed in large scale studies, then clinicians will have to consider with due care and attention the treatment choice in the case of patients affected by cancer and cardiovascular diseases, bearing in mind the possible opposing role of EPCs in these patients (Fig. 1).

In fact, in light of Cardioncology, circulating EPCs could became a double edged weapon for new antiangiogenetic therapies. Whether to target circulating EPCs aiming at the limitation of tumour growth and metastasisation or to spare (and possibly increase) them in order to limit or even improve the cardiac damage exerted by some antineoplastic drugs could be the next dilemma that oncologists and cardiologists will have to face in the forthcoming years.

Conflict of interest statement

None declared.

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