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Significance and therapeutic implications of endothelial progenitor cells in angiogenic-mediated tumour metastasis

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ABSTRACT

Cancer conveys profound social and economic consequences throughout the world. Metastasis is responsible for approximately 90% of cancer-associated mortality and, when it occurs, cancer becomes almost incurable. During metastatic dissemination, cancer cells pass through a series of complex steps including the establishment of tumour-associated angiogenesis. The human endothelial progenitor cells (hEPCs) are a cell population derived from the bone marrow which are required for endothelial tubulogenesis and neovascularization. They also express abundant inflammatory cytokines and paracrine angiogenic factors. Clinically hEPCs are highly correlated with relapse, disease progression, metastasis and treatment response in malignancies such as breast cancer, ovarian cancer and non-small-cell lung carcinoma. It has become evident that the hEPCs are involved in the angiogenesis-required progression and metastasis of tumours. However, it is not clear in what way the signalling pathways, controlling the normal cellular function of human BM-derived EPCs, are hijacked by aggressive tumour cells to facilitate tumour metastasis. In addition, the actual roles of hEPCs in tumour angiogenesis-mediated metastasis are not well characterised. In this paper we reviewed the clinical relevance of the hEPCs with cancer diagnosis, progression and prognosis. We further summarised the effects of tumour microenvironment on the hEPCs and underlying mechanisms. We also hypothesized the roles of altered hEPCs in tumour angiogenesis and metastasis. We hope this review may enhance our understanding of the interaction between hEPCs and tumour cells thus aiding the development of cellular-targeted anti-tumour therapies.

1. Introduction

The endothelial progenitor cells (EPCs) are a subtype of stem cells that reside within a stem cell niche in the bone marrow and are required in some repairing processes, such as

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myocardial ischemia and infarction, limb ischemia and wound healing. In particular they are defined by co-expression of the markers of CD34, CD309 (VEGFR-2/KDR) and CD133. Notably, CD133 is expressed in the most primitive EPCs and lost during maturation to endothelial cells, in which other markers are expressed, such as vascular endothelial cadherin and von Willebrand factor. The frequency of $CD34^+$ cells which also express CD309 and CD133 in peripheral blood in healthy individuals is around $0.4 \pm 0.2\%$ of the total $CD34^+$ population (0.002% of total mononuclear cells). Cord blood and foetal liver-derived $CD34^+$ cells contain $1.4 \pm 0.5\%$ and $1.2 \pm 0.3\%$ $CD133^+/CD309^+$ cells, respectively (Hristov and Weber, 2004; Peichev et al., 2000). In normal physiological conditions EPCs are quiescent but in response to a vascular injury, they acquire the ability to circulate in the peripheral blood, proliferate and differentiate into mature endothelial cells. In response to a gradient of growth factors and cytokines released by the damaged site, EPCs are able to migrate and home to the local endothelium, contributing to neovasculature (Peichev et al., 2000; Asahara and Kawamoto, 2004). There are no systematic studies on the physiological variation of EPCs but it has been proved that their number may varies in pathological conditions. For example, the level of circulating EPCs is lower in diabetic and vascular disease patients than in healthy people (Fadini et al., 2006; Vasa et al., 2001) and rises after an acute myocardial infarction and in some malignancies (Nowak et al., 2010a; Massa et al., 2005). EPCs are activated not only by a vascular injury, but also in the sites where the oxygen level is low such as foetal liver, umbilical cord blood and tumour tissues (Asahara and Kawamoto, 2004). EPC isolation and enumeration is made difficult by the low number of cells in peripheral blood, methodological issues and a lack of consensus on phenotypic identification. Despite this, EPCs can be isolated from peripheral blood through three general approaches and using specific methodologies to enrich their number. In the first approach EPCs are isolated by cultivation of mononuclear peripheral blood cells (PBMCs) in fibronectin-coated plates. After 2–3 weeks, the adherent cells that are able to ingest acetylated low density lipoprotein (acLDL) and to bind specific lectins are classified as EPCs. Circulating EPCs may be also purified using monoclonal antibodies and fluorescence activated cell sorting (FACS). Finally, EPCs may be obtained using two different *in vitro* colony forming assays, the colony forming unit-Hill (CFU-Hill) and endothelial colony forming cells (ECFC) assays (Yoder and Ingram, 1796). In this review, we summarised the clinical relevance of EPCs in cancer. In particular, we evaluated the possible biomarker value of EPCs in cancer, and explored how tumour microenvironment may regulate the activation, mobilisation and homing of EPCs. We also highlighted the internal and external stresses which contribute to the interaction of tumour cells and EPCs. We further proposed possible therapeutic strategies by targeting tumour-associated EPCs to halt tumour angiogenesis and metastasis.

2. Significance of EPCs in angiogenic-mediated tumour metastasis

Although the mechanisms that regulate the migration of cancer cells from the primary to secondary organs have been widely studied, some aspects of this multistep process are still unclear (Hoshino et al., 2015). Stem cells are recruited to the tumour site to enhance new blood vessel formation by secreting angiogenic molecules and/or by trans-differentiation into endothelial-like cells, such as dendritic cell precursors, circulating mesenchymal stem cells (MSCs) and a subset of adult peripheral blood leukocytes functions as endothelial cell progenitors (Coukos et al., 2005; Harraz et al., 2001; Xu and Li, 2014). Haematopoietic precursor cells from the bone marrow, including EPCs, appear to be activated on specific sites before the tumour cells get there, contributing to the formation of a "pre-metastatic niche", in which non-cancer cells promote metastasis development. Changes in the level of circulating EPCs in patients with cancers can indicate treatment response as well as the grade of the malignancy. Thus, the number or functional alteration of EPCs in peripheral blood of cancer patients may be used as a biomarker to evaluate the risk of metastasis and raises the possibility that targeting circulating progenitors may have a therapeutic value.

2.1. Clinical relevance of EPCs in cancer

The condition in which a region of the body is deprived of an adequate oxygen supply is called "hypoxia". During embryonic development, where the level of oxygen is low, EPCs contribute to the physiological vasculogenesis of the embryo (Schmidt et al., 2007). In adults, EPCs can be activated by a vascular injury or hypoxia and play an important role in neo-vasculogenesis, the formation of new vessels during post-natal life (Ceradini et al., 2004). In normal conditions, EPCs are mobilized from the bone marrow in response to paracrine factors released by the injured tissues, such as Vascular Endothelial Growth Factor (VEGF) and Stromal cell-Derived Factor 1 (SDF-1). When the wound is repaired, the physiological state is established again and EPCs are not recruited anymore. In pathologic states such as tumour aggression, when a chronic state of hypoxia and/or inflammation occurs, the EPCs are constitutively activated and play a double role: from one side, they contribute to the tumour sprouting thanks to their ability to form new vessels. On the other side by releasing paracrine secretions of pro-angiogenic growth factors, they contribute to maintaining an inflammatory state, one of the essential biological characteristics for tumour growth and progression (Hanahan and Weinberg, 2011). It has been shown that hypoxia itself is good for growth and survival of stem and progenitor cells, inducing a "protective" autophagy and moderate apoptosis during times of cellular stress. However, if the exposure to low oxygenation in tissues becomes a chronic state, the result is a major apoptosis and destructive necrosis (Sharma and Wu, 2013).

It has been demonstrated that EPCs are mobilized from the bone marrow in different types of malignancies, such as hepatocellular carcinoma, lung, pancreatic and breast cancer (Yu et al., 2007; Nowak et al., 2010b; Starzynska et al., 2013; Ono et al., 2014; Buchanan et al., 2012). Several studies have shown that in different types of cancers, the EPC level is very high in both tissues and peripheral blood of cancer patients compared to those of healthy donors. For instance, the increase of EPCs in peripheral blood of hepatocellular carcinoma patients was observed (Yu et al., 2007). In patients with Non-Small-Cell Lung Carcinoma (NSCLC) and Small Lung Carcinoma (SLC) the number of circulating EPCs increases in proportion to the disease progression, and there appears a correlation between circulating EPC concentration and the stage of the malignancy (Nowak et al., 2010b). If further studies can confirm this finding, it may be possible to consider the EPCs from peripheral blood as non-invasive biomarkers for the monitoring of tumour progression, in concert with other tumour-specific biomarkers already available. Studies also suggested a possible application of circulating EPCs as predictors of the malignancy grade of some tumours. In fact, a decrease of circulating EPCs has been observed in patients who respond well to anticancer treatments. Circulating EPCs have also been proposed to be prognostic and predictive biomarkers for gastric cancer patients treated with chemotherapy (Ahn et al., 2010). Indeed there is a reduction of EPC levels in breast cancer patients after cytotoxic therapy (Kuo et al., 2012). The level of EPCs in blood can be distinguished from healthy donors, NSLC patients who responded well to the treatment (chemotherapy/radiotherapy) and NSLC patients who did not

respond to treatment (Dome et al., 2006). The same group also showed that there was a higher incidence of death in patients who had higher EPC levels in blood before the treatment, compared with those with lower level of EPCs. This observation strengthens the hypothesis that EPC levels in NSCLC may correlate with tumour burden (Dome et al., 2006). However, the clinical relevance of EPCs can be complicated depending on particular treatments in lung cancer. For instance, some chemotherapeutic agents may induce EPC mobilisation from the bone marrow to the tumour site, supporting the tumour spread. And although a combination of anti-angiogenic drugs may amplify the effect of some anti-cancer therapies, in a few cases the opposite effect has been reported (Shaked et al., 2008).

2.2. EPCs in cancer diagnosis and prognosis

EPCs are required for the regulation and protection of endothelia, as well as the formation of new vessels. Consequently, their number increases in response to chemokines released in the microenvironment after a trauma or when a condition of chronic inflammation occurs. Numerous studies have focused on EPCs in peripheral blood to evaluate whether a variation in their number could represent a tool to predict a pathological state. It is well known that in patients affected by different diseases the number of circulating EPCs may vary within patients affected by the same pathology and between patients and healthy people. Moreover, in some cases a correlation of circulating EPCs with clinical outcomes has been proposed. For example, low levels of EPCs have been observed in diabetic and vascular disease patients. Diabetes is characterised by hyperglycaemia and impaired endothelial function. High glucose levels result in a reduction of endothelial nitric oxide synthase (eNOS) activity and nitric oxide (NO) availability which is important both for the mobilisation and function of EPCs. Therefore there is a reduction of circulating EPCs when glucose in blood is too high. It has also been observed that EPCs proliferate at a very low rate in diabetes patients and their number is significantly reduced (Tepper et al., 2002; Fadini et al., 2007; Hamed et al., 2009). Low level of circulating EPCs seem also to correlate with complications associated with diabetes, such as peripheral arterial disease (Fadini et al., 2006).

A decrease in EPC numbers is also associated with the pathogenesis of vascular complications. EPC number and migratory capacity are impaired in patients with coronary artery disease (Vasa et al., 2001). It has been shown that reduced levels of circulating EPCs may predict future cardiovascular events, suggesting a possible role of these types of cells as biomarkers (Schmidt-Lucke et al., 2005). On the other hand, an increase in EPC numbers in the peripheral blood of patients with acute lung injury is associated with improved survival (Burnham et al., 2005).

As reported previously, in tumours EPCs are mobilized from the bone marrow to contribute to the formation of new vessels and, mainly for this reason, the levels of EPCs in the blood of cancer patients is higher than in healthy people (Arbab et al., 2006). It has been shown that EPCs in peripheral blood may represent a useful diagnostic and prognostic tool to monitor the clinical state of patients. In NSCLC a higher level of EPCs correlates with a poor overall survival. NSCLC patients who achieve a partial or complete remission after anti-cancer therapy show lower level of circulating EPCs than patients with stable or progressive disease, suggesting that the number of EPCs correlates with the treatment efficacy in this malignancy (Dome et al., 2006). Furthermore it has been shown that patients with more advanced stages of both NSCLC and SCLC have higher levels of circulating EPCs than patients at a lower stage probably due to the larger tumour size and the presence of distant metastases (Nowak et al., 2010b). One study has shown that, in response to some chemotherapy regimens, the number of EPCs in the plasma of cancer patients continues to increase independently from the level of cytokines (Roodhart et al., 2010). So far some hypotheses have been proposed for the use of EPCs as biomarkers to monitor the progression of the disease and to discriminate between a good and bad response to therapies. It has been demonstrated that EPCs may represent a good marker to evaluate the response of colorectal cancer patients to antiangiogenic drugs (Matsusaka et al., 2011; Ronzoni et al., 2010). Although preclinical studies have been shown their usefulness for the treatment of some malignancies, the role of antiangiogenic drugs is controversial especially because their effects are limited by drug resistance (Shaked et al., 2008). In breast cancer patients with a relatively high amount of EPCs, chemotherapy reduces the number of circulating endothelial cells, which are probably detached from the pre-existing vessels. However, at the same time, it induces the mobilisation of the EPCs from the bone marrow. This evidence strengthens the hypothesis that EPCs may contribute to tumour-associated angiogenesis and, consequently, the beneficial effects of an early antiangiogenic therapy in combination with chemotherapy (Fürstenberger et al., 2006).

As shown in Table 1, elevated EPC number can be frequently observed in some cancers and their relevance for the evaluation of the stage of a tumour has been proposed. For instance, the number of EPCs in both cancer-adjacent and cancer tissues of patients with late-stage gastric cancer was lower than in early-stage patients. Thus the EPC number may represent a diagnostic index although the role of EPCs in this malignancy has not been well characterised (Ha and Kim, 2014).

The preliminary data on the possible use of circulating EPCs as a non-invasive biomarker is promising. They may be used in concert with other molecules instead of some invasive prognostic markers available, such as microvessel density (MVD). For instance, in some malignancies not only EPC but also VEGF and haematopoietic progenitor cells (HPCs) levels were found higher in patients than in controls and all of them may be considered to monitor the progression of the disease (Nowak et al., 2010a; Jain et al., 2012). Nevertheless EPC number may be affected by other conditions, such as cardiovascular diseases (Bahlmann et al., 2004; Friedrich et al., 2009). For this reason further studies are necessary to validate the use of EPCs as biomarker in cancer.

3. Interaction of EPCs and tumour cells in aggressive tumour microenvironment

The tumour microenvironment has been recognised as the product of a developing crosstalk between different cell types. The direct and indirect interactions between cancer cells, EPCs, inflammatory immune cells and endothelium support the growth and spread of tumours. In the following paragraphs the consequences and mechanisms underlying the alterations of tumours and EPCs in aggressive tumour microenvironment are described.

3.1. Effect of tumour microenvironment on EPCs

As reported previously, in the cases of ischemia and tumour, EPCs are mobilised from the bone marrow and hypoxia plays an important role in their homing. Pathologic hypoxia is the condition that induces the formation of new capillaries from pre-existing vessels enabling the oxygen supply to the tumour mass, one of the crucial characteristics for cancer cell survival (Hanahan and Weinberg, 2011). The oxygen tension regulates the transcription activator hypoxia inducible factor (HIF), a dimer formed by two subunits (HIF1-α and HIF1-β). In normoxic conditions HIF1-α is degraded in the proteasome. Conversely, in hypoxia the two subunits are able to combine together and translocate into the nucleus, where they activate the transcription of a large number of genes required for tumour progression, such as vascular

Table 1
Increase of circulating endothelial progenitor cells (cEPCs) in some cancers.

Cancer	Treatment	cEPCs	Correlation with the stage	Reference
Liver	None	Higher in patients than in healthy people	–	(Yu et al., 2007)
Lung (NSCLC and SCLC)	Various chemotherapeutic regimens	Higher in patients than in healthy people	Yes	(Nowak et al., 2010a)
Lung (NSCLC)	Gemcitabine and cisplatin (plus radiotherapy in some cases)	Higher in patients than in healthy people	Yes	(Dome et al., 2006)
Breast colorectal, ovarian, esophagus, prostate, head and neck, sarcoma, cervix	Various chemotherapeutic regimens	Higher after the first cycle of chemotherapy	–	(Roodhart et al., 2010)
Breast	Anthracycline and/or taxane based chemotherapy	Higher after chemotherapy in patients with low EPCs	–	(Fürstenberger et al., 2006)

endothelial growth factor-A (VEGF-A), platelet-derived growth factor (PDGF), erythropoietin, stromal cell-derived factor (SDF1-α) and C-X-C chemokine receptor type 4 (CXCR 4) (Paliege et al., 2010; Tang et al., 2016; Schioppa et al., 2003). The loss of function of HIF as a dimer inhibits EPC proliferation and differentiation, thus reduces their ability to form new vessels (Branco-Price et al., 2012; Jiang et al., 2006). The knock-down of HIF1-α in EPCs has beneficial effects, not only on pathologic angiogenesis but also on the remodelling of blood vessel which return to their regular shapes (Du et al., 2008).

Another common feature of neoplastic diseases is the ability of cancer cells to avoid the immune response and maintain a chronic state of inflammation (Hanahan and Weinberg, 2011). During the inflammation process, cytokines are released from the damaged or hypoxic tissue, producing a molecular gradient which guides EPCs, pericyte progenitors and CD45+ vascular modulatory cells from the bone marrow to the inflamed tissue (Ahn and Brown, 2009). The up-regulation of cytokines and some interleukins produced by the cancer tissue itself and by macrophages is a common condition in cancer, where the inflammation persists (Mosser and Edwards, 2008). However, cytokines have a crucial role not only in the maintenance of a malignant microenvironment but also in survival, growth, mutation, proliferation and differentiation of both tumour and stromal cells (Balkwill and Mantovani, 2001).

One of the most widely studied cytokines using *in vitro* and *in vivo* models is VEGF, which is overexpressed in different types of malignancies. VEGF acts through three tyrosine kinase receptors (VEGFR1, 2 and 3), two of which are specifically involved in angiogenesis (VEGFR1 and VEGFR2) (Ferrara et al., 2003; Senger et al., 1994; Gerber et al., 1998). VEGF has various effects on endothelial cells, including the increase of vascular permeability, facilitation of survival *via* reducing cell apoptosis and the support of tumour tissue adhesion by reshaping the extracellular matrix of the malignant cells (Senger et al., 1994; Pidgeon et al., 2001). It has been demonstrated that in tumours, a high plasma level of VEGF promotes mobilisation of EPCs from the bone marrow (Lyden et al., 2001a). Moreover, VEGFR1-deficient mice show several vascular malformations, suggesting the importance of this cytokine in vascular remodelling. Therefore, the effect of VEGF on EPCs is to stimulate their mobilisation from the bone marrow and their proliferation. VEGF can improve the proliferation of EPCs by increasing the level of NO following the activation of the phosphatidyl-inositol-3-kinase (PI3K)-Akt/eNOS signalling pathway (Chen et al., 2011a). NO is an ubiquitous intercellular messenger, and in blood vessels its synthesis is controlled by the endothelial enzyme eNOS (Knowles and Moncada, 1992). In physiological conditions, NO contributes to the maintenance of vascular tone and blood pressure (Calver et al., 1992). When the level of oxygen becomes lower, the increase of eNOS by stem cells from the bone marrow promotes migration and proliferation of these cells and tubule formation (Branco-Price et al., 2012; Lu et al., 2014). NO stimulates matrix metalloproteinase 9 (MMP-9) that activates the detachment between EPCs and the bone marrow niche through the cleavage of the membrane Kit ligand (mKitL). mKitL is converted to the soluble form (sKitL) and binds the EPC membrane, leading to their release into the bloodstream.

VEGF also regulates angiogenesis together with two paracrine factors, angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2). Ang-1 is expressed in many adult tissues and Ang-2 is primarily produced by endothelial cells at the site of active vascular remodelling (Huang et al., 2010). Both bind the tyrosine kinase receptor Tie-2 in an antagonistic manner. Ang-1 promotes vessel maturation, integrity and vascular quiescence. Ang-2 causes the dissociation of pericytes from the tumour vasculature, promoting instability of blood vessels (Maisonpierre et al., 1997; Fagiani and Christofori, 2013). During the first stages of neoplastic transformation, VEGF and Ang-1 promote the mobilisation of EPCs *via* remodelling the BM vascular architecture (Hattori et al., 2001). When VEGF levels are high, VEGF stimulates the release of Ang-2 by endothelial cells in the interior tumour vessels, reducing the signal for vessel regression by Ang-1 competing for the binding to Tie-2 receptor. It has been shown that VEGF and Ang-2 act in a synergistic way to promote angiogenesis. In fact, the depletion of both VEGF and Ang-2 activity results in a significant inhibition of tumour growth and EPC recruitment (Kienast et al., 2013; Visconti et al., 2002).

As mentioned, a chronic state of hypoxia produces major apoptosis and necrosis, contributing to the maintenance of an inflammatory environment (Shaked et al., 2008). Some pro-inflammatory cytokines, such as interleukins and tumour necrosis factor (TNF-α) are involved in tumour growth and progression, and have effects on EPCs (Voronov et al., 2014). In particular, TNF-α is able to reduce EPC proliferation, migration and tubule formation capacity in a dose- and time-dependent manner (Chen et al., 2011b).

3.2. Mechanisms by which tumour cells modulate the behaviour of EPCs

EPCs participate in tumour progression due to their ability to form new vessels and secrete paracrine factors (Gao et al., 2008a). Both their physiological and acquired pathological characteristics result from signals coming directly from the tumour cells and indirectly from cells modified by the tumour and/or microenvironment.

Hypoxia alters the gene expression of different types of cells, changing their morphology and properties. For instance the tumour-associated macrophages (TAMs) play a crucial role in cancer (Lewis et al., 1999) and their high density is correlated with a poor patient prognosis (Bingle et al., 2002). TAMs and other cancer-activated cells are able to influence EPC behaviour by secreting growth factors and cytokines, such as Transforming Growth Factor β (TGF-β), Tumour Necrosis Factor α (TNF-α) and interleukins (Locksley et al., 2001; Lewis and Pollard, 2006).

TGF-β is a family of cytokines that regulate many aspects of cellular behaviour, such as proliferation, differentiation and apoptosis. TGF-β members regulate cell responses by binding the serine/threonine kinase Smad receptors or through different Smad-independent pathways (Derynck and Zhang, 2003). The interaction between TGF-β ligands and their receptors activates the downstream effectors, which transmit the signals from the cell membrane to the nucleus and result in subsequent transcription of target genes (ten Dijke et al., 2000). In physiological conditions TGF-β acts as a tumour suppressor, but during neoplastic transformation it promotes cell growth, motility and invasion. TGF-β1 is the member of TGF-β family which is specifically involved in angiogenesis. Considerable studies have shown that TGF-β1 is able to influence EPC behaviour (Imamura et al., 2010; Sales et al., 2006; Evrard et al., 2012). Endothelial cells express two TGF-β receptors, ALK1 and ALK5, which act in an antagonistic way (Goumans et al., 2002). The TGF-β/ALK1 signalling activates the transcription of the Inhibitor of DNA Binding (ID) protein molecules. In particular, ID-1 is highly expressed in EPCs and promotes the proliferation and migration of EPCs through P13K/Akt/NFκB/survivin signalling cascades (Gao et al., 2008a; Li et al., 2012). Conversely, when TGF-β binds ALK5 receptor, it stimulates the transcription of the plasminogen activator inhibitor 1 (PAI-1), which prevents vascular cell migration and angiogenesis (Siegel and Massague, 2003). The different use of the two receptors by TGF-β may explain the double roles of this cytokine in endothelial cells and EPCs. The high expression of ID-1 in EPCs suggests that the preferential use of ALK5 receptor by TGF-β may be one of the strategies adopted by the tumour for its expansion.

In normal conditions the EPCs are quiescent and reside in their BM niche in close association with haematopoietic stem cells and stromal cells. Notch is one of the key components of the BM niche. It is a transmembrane protein that is involved in many aspects of differentiation and cell fate determination. Perturbation of the Notch pathway is linked to a spectrum of diseases including cancer (Louvi and Artavanis-Tsakonas, 2006; Rampias et al., 2014; Cheng et al., 2014a; Espinoza and Miele, 2013). In Mammals, the canonic Notch signalling involves four Notch receptors (Notch1-4) and six Notch ligands (Jagged1-2, Delta1, 3, 4 and X-Delta2) (D'Souza et al., 2008). When a Notch receptor interacts with its adjacent ligand or on the surface of an opposing cell, the receptor undergoes two proteolytic cleavages and the activated form of Notch (NICD-Notch Intracellular Domain) is released into the cell. NICD translocates into the nucleus, leading to transcriptional activation of target genes. Notch is required for the maintenance of the quiescence of the stem cells in the bone marrow, including EPCs. One of the activators of Notch signalling is Tumour Necrosis Factor (TNF), secreted mainly by the macrophages. A tumour state can induce the modification of macrophages that secrete TNF and other cytokines, promoting the overexpression of Notch receptors on the cell surface of osteoblasts (Varnum-Finney et al., 2003; Fernandez et al., 2008; Kwon et al., 2009). The interaction between Notch ligands on the osteoblasts and Notch receptors of the EPCs permits the activation of EPCs. Notch signalling activation in progenitors is mainly a result of Jagged1 activity. In fact, it has been demonstrated that the loss of Jagged1 expression leads to a reduced endothelial lineage gene expression in BM progenitors, lower EPC colony formation, reduced migration and loss of the ability of EPCs to form new vessels (Kwon et al., 2008).

TNF-α is a master regulator of inflammation and is produced by tumour and inflammatory cells. In tumours it can promote tumour progression by supporting angiogenesis or induce the apoptosis of cancer cells. TNF-α has been shown to act as both a positive and negative regulator of haematopoietic progenitor cells (Cuturi et al., 1987; Caux et al., 1993). The divergent effects of TNF-α depend on its concentration, duration of exposure (Fajardo et al., 1992) and type of targeted cells (Balkwill, 2009). Actually at low concentration TNF-α promotes angiogenesis by upregulating the levels of VEGF, bFGF and IL-8. In contrast, when its concentration becomes higher, it prevents the formation of new vessels (Fajardo et al., 1992; Caporali et al., 2008). The different roles of TNF-α can be attributed to its ability to bind two distinct receptors: p55/TNFR1 and p75/TNFR2. TNF-α/p55 signalling increases the expression of some genes that inhibit the apoptotic effect of TNF-α acting *via* the NF-κB pathway. On the other hand, TNF-α/p75 mediates apoptosis and cell cycle arrest. The role of TNF-α in EPCs is to promote their survival, migration and incorporation into nascent vessels by binding the p75 receptor (Jacobsen et al., 1994). An *in vivo* study clearly demonstrated that TNF-α/p75 signalling induces the apoptosis of EPCs in p75KO mice (Sasi et al., 2011). It can be concluded that the main effect of TNF-α on EPCs during neoplastic transformation is to reduce their number by inducing apoptosis. Moreover, TNF-α enhances the expression of VCAM, ICAM, P-selectin and E-selectin, adhesion molecules in endothelial cells which supports the EPC adhesion in the hypoxic sites (Gong et al., 2011).

Some studies have shown the role of interleukins (ILs) in survival, proliferation and differentiation of hematopoietic progenitor/stem cells. For instance, IL-1β is a potent immunoregulatory and proinflammatory cytokine secreted by the activated immune cells. IL-1β increases the number of EPCs, their viability, proliferation and capacity to form new vessels *in vitro* acting through ERK-MAPK signalling (Rosell et al., 2009). Another study shows that IL-1β upregulates VEGF-A production *in vitro* through the phosphatidyl-inositol-3kinase-Akt signalling pathway (Yang et al., 2012), enhancing the effects of EPCs in tumour spread. Moreover, in malignancies, cross-talk between EPCs and activated monocytes occurs: after EPC activation and mobilisation, EPCs themselves are able to increase IL-1β production in monocytes, contributing to the maintenance of a pro-inflammatory environment (Zhang et al., 2013). During neoplastic transformation, normal endothelial cells (NECs) are modified into tumour-derived endothelial cells (TECs) and play a key role in EPC differentiation, thanks to their acquired capability to release the cytokine IL-3 into the tumour microenvironment. IL-3 induces the expression of adhesion molecules in TECs, such as mKitL, through the Akt pathway. In angiogenic sites, mKitL acts as an adhesion receptor for EPCs, interacting with c-Kit receptors of EPCs (Dentelli et al., 2011). So, IL-3 promotes both the recruitment and adhesion of EPCs in inflammation sites. Furthermore, a microenvironment containing IL-3 stimulates BM-derived angiogenic cell proliferation and specification *via* a STAT5-mediated pathway (Zeoli et al., 2008). Finally, it has been shown that IL-6 plays a role as a pro-angiogenic cytokine. EPCs possess IL-6 receptors and a previous study demonstrates that IL-6 is able to bind its receptor on EPCs and contributes to angiogenesis through the activation of both REK-1/2 and STAT3 pathways (Fan et al., 2008).

3.3. *Effects of EPCs on cancer invasion in tumour microenvironment*

Endothelial cells and the cells of the immune system promote the directional movement and adhesion of EPCs to neovessels due to their ability to secrete paracrine factors, for which EPCs have receptors. In cancer, the cross talk between EPCs, inflammatory cells and the endothelium supports the formation of the ideal microenvironment for tumour spread. Although the mechanisms that regulate EPC mobilisation have been well described (Cui and Madeddu, 2011), the contribution of EPCs to the metastatic process has not been completely characterised. In tumours the chronic state of hypoxia makes the cells constitutively active. HIF1-α induces the release of stromal cell-derived factor 1 (SDF-1) chemokine from the tumour cells, endothelium, stromal fibroblasts and platelet gran-

ules (Ceradini et al., 2004; Mohle et al., 1998; Orimo et al., 2005; Jin et al., 2006). The receptor of SDF-1 is CXCR4, which is widely expressed in the differentiated EPCs. In the first stages of neoplastic transformation the gradient of SDF-1, together with the high concentration of eNOS, attracts progenitor cells toward the hypoxic tissue. This may represent one of the strategies adopted by the cancer to maintain a good pool of cells for the generation of new vessels. During the tumour progression, EPCs start to secrete SDF-1 and VEGF-1 spontaneously (Urbich et al., 2005). Tumour cells and mature endothelial cells express CXCR4 on their cell surface, so the gradient of SDF-1 produced by the EPCs may promote the extravasation and development of a pre-metastatic site before the arrival of the malignant metastatic cells (Kaplan et al., 2005; Jin et al., 2012). In addition, the indirect interaction between the resident fibroblasts and BM-derived progenitor cells participates in the formation of the pre-metastatic niche (Kaplan et al., 2005). In fact, the BM-derived progenitor cells, including EPCs, express integrin α4β1 and VEGFR1. The growth factors released into the microenvironment up-regulate fibronectin in fibroblasts, which is one of the ligands of α4β1, contributing to their directional migration. Based on this experimental evidence, it has been demonstrated that the use of antibodies against CXCR4 improves the anti-tumour effect of chemotherapy, and the knock-out of VEGFR1 in EPCs reduces tumour metastasis development (Hassan et al., 2009). SDF-1 regulates the trafficking of progenitor cells from and to the bone marrow. If the cells of the immune system produce SDF-1, EPCs may be attracted to distant sites and start to produce SDF-1 spontaneously, promoting the spread of the tumour in distant sites. The existence of a pre-metastatic site is consistent with the seed and soil theory postulated by Paget in 1889 and one of the hypotheses is that EPCs may play a crucial role in its formation.

Some evidence suggests an active role for EPCs in cell migration and invasion also due to their ability to activate MMP-9 (Shih et al., 2014), an enzyme responsible for disintegration of the basement membrane. The subsequent release of Kit-ligand and VEGF-A in the microenvironment supports the formation of a metastatic niche. Moreover, ID1 and ID3 inhibition prevents metastatic growth, suggesting a crucial role for these proteins in metastasis (Gao et al., 2008a; Kaplan et al., 2005). The up-regulation of MMP-9 by SDF-1 normally occurs in the bone marrow for the mobilisation of progenitor cells (Petit et al., 2007) and cancer uses the same mechanism to support its spread. Considering these facts, it is possible that SDF-1 facilitates cell egress not only during the physiological processes but also in pre-metastatic niche development (Fig. 1).

4. Roles of internal and external stresses in interaction of tumour cells and EPCs

Cells are affected by many internal and external stimuli, some of which may induce stresses resulting in damage to the structure or function of proteins, DNA or other essential macromolecules. The capacity of the cells to respond to stress depends on the proteome expressed at a particular time and is species- and cell type-dependent (Kültz, 2005). In response to stress a cell can re-establish cellular homeostasis or adopt an altered state (Milisav, 2011). During the development of human tumours, cells acquire some characteristics allowing them to survive in different environments. In response to external stimuli cancer cells change their morphology and gene expression by direct alteration of DNA or modifications in epigenetic regulation.

The typical stresses involved in neoplastic transformation can be categorised as oncogenic, genotoxic and non-genotoxic. Oncogenic stress occurs when there is activation of anti-oncogenic pathways,

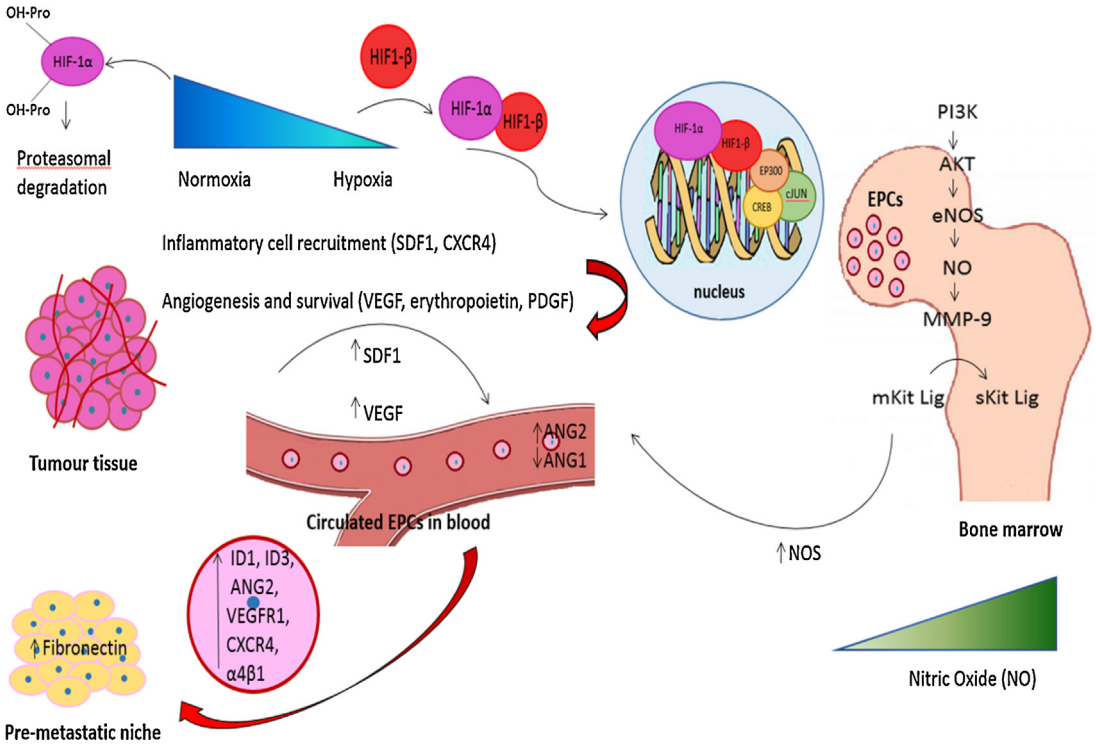

Fig. 1. Endothelial progenitor cells (EPCs) activation in tumour microenvironment. The chronic state of hypoxia (blue) in the tumour microenvironment leads to the constitutive transcription of paracrine factors which stimulate the activation and release of the EPCs from the bone marrow through the PI3K/AKT signalling pathway. PI3K/AKT cascade activates the nitric oxide synthase enzyme (NOS) increasing the level of nitric oxide (NO) (green) which induces the release of progenitor cells, including EPCs, into circulation. In the bloodstream EPCs home to the hypoxic sites with response to pro-angiogenic cytokines secreted by tumours. Activated EPCs express and secrete paracrine factors in return, which increase the vessel permeability thus promote the metastasis of malignant tumours. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

due to the inactivation of tumour suppressor genes and/or the activation of oncogenes. Non-oncogenic stress is induced by carcinogens that can be classified as genotoxic and non-genotoxic. Genotoxic carcinogens (UV rays, ionizing radiation, drugs, and chemicals) form complexes with DNA leading to various type of damage such as removal of bases or formation of cross-links between the two helices. On the other hand, the non-genotoxic carcinogens (hypoxia, temperature, growth factors and viruses) damage DNA by disrupting cellular structure, changing the rate and regulation of cellular processes (Lee et al., 2013).

In response to one of the stresses reported above, the first strategy for preventing the proliferation of potential cancer cells is to arrest cell growth. This process is called "senescence" and can be induced by different triggers. Primary cells are not able to proliferate indefinitely, mainly due to the attrition of telomerases and reach "replicative senescence" (Hayflick, 1998). Certain types of stress triggers, such as DNA damage, oncogenic stress and alterations in the epigenetic regulation, produce stress-induced premature senescence (SIPS) (Serrano and Blasco, 2001). Given that cancer is an age-related disease, it is reasonable to consider cellular senescence as an important mechanism for both preventing and promoting tumourigenesis, depending on aging. It was demonstrated that in young epithelial tissues the environment suppresses the expression of potential neoplastic phenotypes. With age, senescent cells of the basement stroma accumulate and secrete factors, such as cytokines and growth factors that contribute to tumour progression (Campisi, 2003). As reported previously, EPCs contribute to the maintenance of a chronic state of inflammation by increase necrosis. It has also been demonstrated that a prolonged inflammatory stimulation increases senescence in EPCs (Zhang et al., 2009) and it is possible that both senescence and necrosis of EPCs contribute to tumour survival. The role of necrosis in cancer has been established; in particular in EPCs it may contribute to the support of the ideal microenvironment for the tumour spread. On the other hand, it is possible that a small proportion of EPCs attempt to protect themselves from tumour progression by activating the apoptotic pathway. However further investigations are needed to better understand the precise contribution of these two processes in both cancer cells and EPCs during malignant progression.

The DNA-damage response (DDR) is a system adopted by cells to detect DNA lesions, signal their presence and promote repair. The inability to repair DNA damage can result in cell cycle arrest, senescence or apoptosis, leading to various disorders including cancer. In response to DNA damage both ataxia telangiectasia mutated (ATM) kinase and DNA dependent protein kinases are activated to prevent the proliferation of potential malignant cells. Senescence can be induced especially by alterations in telomere length. The telomere-binding protein TRF-2 plays a central role in telomere maintenance and protection against end-to-end fusion of chromosomes (Karlseder et al., 1999). It has been shown that the overexpression of TRF2 leads to a "juvenation" of EPCs by delaying replicative senescence and improving their migration ability. In this case TRF2 protein might correlate with cellular aging since older EPCs assume the same age characteristics of the younger cells, such as the ability to migrate to distant sites (Assmus et al., 2003; Spyridopoulos et al., 2004). EPC senescence may be associated also with telomerase activity. As reported above, the PI3 K/AKT signalling pathway plays an important role in EP metabolism through increasing NO levels, which subsequently activates telomerase and delays EPC senescence. Positive regulators of the PI3 K/AKT signalling pathway, such as statin, VEGF, HDL and oestrogen lead the upregulation of human telomerase reverse transcriptase (hTERT) at the transcriptional level and phosphorylated hTERT that can trigger the telomerase activity post-transcriptionally (Yang et al., 2008). Nevertheless, some evidence suggests that high levels of ROS maintain Akt in the dephosphorylated state, leading to a decrease in telomerase activity and accelerated EPC senescence (Yang et al., 2008). A plausible hypothesis of these observations is that, in physiological conditions, the activation of the PI3K/AKT signalling pathway contributes to the maintenance of the integrity of EPCs, leading them to repair the injury. Then, when ROS increases in response to stress, the senescence of EPCs may rise.

p16INK4a and p19ARF are two proteins with anti-proliferative activity and are transcribed from two different loci on chromosome 9 in humans. p16INK4a is a cyclin-dependent kinase (CDK) inhibitor that promotes cellular senescence in response to multiple stressors, including oncogene activation, telomere erosion and reactive oxygen species (Ito et al., 2006; Carlos et al., 2013). p16INK4a binds cyclin-dependent kinase 4/6 (CDK4/6) and inhibits its activity, which prevents the phosphorylation of retinoblastoma protein (Rb) and stops the cell cycle in S/G1 phase. On the other hand, p19ARF stabilizes the onco-suppressor protein p53 by attenuating the activity of Mdm-2. In human tumours, p16INK4a is expressed at high levels and escape from these protective mechanisms is one of the strategies adopted by the tumour for its survival (Romagosa et al., 2011). In EPCs prolonged inflammatory stimulation by TNF-α increases both the expression of p16INK4a and cellular senescence independently of changes in telomere length. The overexpression of p16INK4a is induced *via* the p38 MAPK pathway, one of the downstream effectors of ras signalling (Zhang et al., 2009).

External and internal stimuli may also cause alterations in the epigenetic maintenance mechanisms of both EPCs and tumour cells. For example, the concentration of glucose is generally lower in tumours than in normal tissues. A "glycolytic switch" in cancer cells occurs: even in the presence of oxygen, cancer cells prefer anaerobic glycolysis rather than oxidative phosphorylation *via* the tricarboxylic acid (TCA) cycle for energy production (Warburg effect) (Cairns et al., 2011; Hirayama et al., 2009). Increased glycolysis produces higher ATP and NADPH levels, making the environment more acidic and contributing to the up-regulation of oxidative stress. Sirtuin-1 (SIRT1) is a protein deacetylase active upon calorie restriction (Cohen et al., 2004). In response to DNA damage or oxidative stress SIRT1 has a repressive effect on tumour suppressor p53 and other transcription factors (Luo et al., 2001; Motta et al., 2004) thus preventing premature senescence. It has been demonstrated that either down-regulation of SIRT1 expression or re-expression of its direct binding partner, caveolin-1, restores ROS-induced acetylation of p53 and premature senescence (Volonte et al., 2014). A reduced number of EPCs has been observed in patients affected by diabetes, where the level of glucose is high (Rosso et al., 2006). A downregulation of SIRT1, due to the high level of glucose, correlates with the activation of the transcriptional activity of the Forkhead transcription factor Fox01 and leads to a reduced level of EPCs in patients affected by diabetes (Balestrieri et al., 2008). Notably, SIRT1 is also upregulated by ROS alone (Wang et al., 2015). Fox01 is a transcriptional factor that negatively controls cell cycle progression and induces apoptosis in response to stress (Huang et al., 2006; Chen et al., 2009; Brunet et al., 1999). When the glucose level is low, as in cancer, the activation of SIRT1 correlates with the decrease of Fox01 levels. Fox01 activity is also impaired by the over-activation of the PI3K/AKT signalling pathway. When stress occurs, the pathological inactivation of Fox01 represents a cell survival signal. Moreover, in response to ROS, EPCs secrete paracrine factors that protect the mature endothelial cells from stress-induced apoptosis, supporting angiogenesis (Yang et al., 2010).

Senescence in EPCs is regulated also by a class of small non-coding RNAs called microRNAs (miRNA or miR). miR expression can vary according to internal and external stimuli and their deregulation is associated with a variety of diseases (Manca et al., 2011; Garbett et al., 2015; Leung and Sharp, 2010; Roufayel et al., 2014; Taki et al., 2014). It has been shown that the down-regulation of

miR22, miR10 and miR21 in aged EPCs suppresses senescence and promotes proliferation (Zhu et al., 2013; Zheng and Xu, 2014). The up-regulation of miR10b and miR196b in EPCs induced by VEGF and the release of microvesicles containing the pro-angiogenic miR126 and miR296 from EPCs correlate with the increase of tumour vasculature (Plummer et al., 2013; Cantaluppi et al., 2012).

The therapies administered to patients affected by cancer represent another stimulus that may have effects both on cancer cells and EPCs. Chemotherapeutic regimens involve the administration of genotoxic compounds that induce cancer cell death. However, this type of therapy kills most of the cells in a tissue but is not tumour-cell specific. Some cancer cells may survive and divide again to develop a new tumour state. In fact not all patients with the same treatment have similar outcomes, some may be therapy-resistant and relapse. The reasons for different responses to the same therapy can be understandable if we consider cancers as a heterogeneous group of diseases. One of the most ambitious aims of "personalised medicine" is to find new patient-specific therapies. In any case, drugs represent an external stimulus that may have different effects on the fate of both cancer cells and EPCs. It has been demonstrated that certain drugs can rapidly induce EPC mobilisation and tumour homing whereas others do not (Shaked et al., 2008). Furthermore it has been suggested that EPC release after treatment is part of a host response mediated by the up-regulation of various cytokines involved in progenitor cell recruitment, such as SDF-1α (Roodhart et al., 2010; Sakamori et al., 2012). Platelets activated by cytokines are one of the main sources of SDF-1α. In particular, they contain VEGF, TSP-1 and SDF-1, therefore blocking their aggregation by targeting Platelet-Derived-Growth Factor (PDGF) may results in a reduction of the number of EPCs at the tumour site and, consequently, a reduction of the formation of new vessels. This could in part explain why the addition of anti-angiogenic therapies enhances the anti-tumour effect of chemotherapy. However, the effect of other therapies is to induce EPC mobilisation which contributes to tumour recovery, especially when administered near the maximum tolerated dose (MTD) (Shaked et al., 2008; Rafii et al., 2008) (Fig. 2).

5. Therapeutic strategies by targeting tumour-associated EPCs

EPC mobilisation may contribute to tumour angiogenesis and formation of metastasis. It has been demonstrated that blocking EPC mobilisation inhibits vasculogenesis and impairs the formation of macro-metastasis *in vivo* (Moccia et al., 2015; Lyden et al., 2001b; Gao et al., 2008b). Blocking genes involved in the homing of EPCs to tumour vasculature may carry the potential for improving antiangiogenic and antitumor effects. Another anti-tumour strategy could be to arrest the mobilisation of EPCs from the bone marrow by inhibiting some of the factors involved in their recruitment. The main regulator of EPC mobilisation is the chemokine SDF-1. The disruption of SDF-1 receptor CXCR4 is essential for the egress of progenitor cells from the bone marrow into the circulation. Four major classes of CXCR4 antagonists and agonists have been described: small peptide antagonists and agonists, non-peptide CXCR4 antagonists, antibodies to CXCR4, and modified antagonists and agonists for SDF-1α (Burger and Peled, 2009; Liles et al., 2003; Abraham et al., 2007). The effect of all these compounds is to prevent the gradient of chemokines that allows the homing of EPCs to the tumour site.

VEGF is another factor involved in EPC mobilisation. Most anti-angiogenic therapies are based on inhibiting the binding of VEGF to VEGFR using neutralising antibodies against the ligand or the receptor, soluble receptors, small molecule inhibitors or therapies

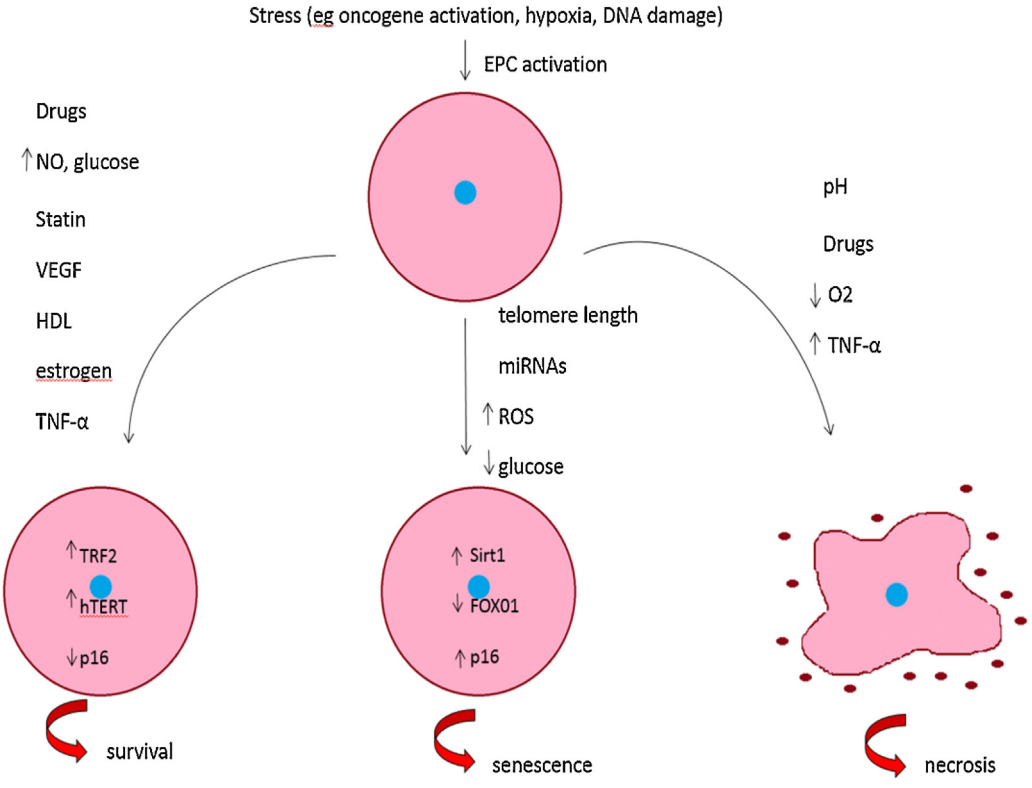

Fig. 2. Effects of stress on EPCs. EPCs are able to respond to external and internal stresses modifying their genetic and epigenetic regulation. In physiological conditions EPCS are able to prevent the proliferation of potential abnormal cells arresting their growth (senescence). During the tumour progression, EPCs acquire the ability to escape the senescence, promoting their survival or undergoing to necrosis depending on crosstalk of a variety of factors released from the tumour microenvironment. Necrotic EPC cells explode and release pro-inflammatory factors that support the tumour microenvironment.

directed against the tyrosine kinase activity of VEGF receptors. It has been shown that several VEGF-targeted drugs, administered either as single agents or in combination with chemotherapy, have beneficial effects on tumour patients (Miller et al., 2007; Russo et al., 2015; Barr et al., 2015; Heng et al., 2009). Pre-clinical studies have demonstrated that the disruption of the VEGF signalling pathway negatively modulates EPC-supported vasculogenesis (Qi et al., 2013; Kerbel and Folkman, 2002). However, some clinical evidence has revealed that EPCs are also implicated in conferring resistance against vascular disrupting agents. Inhibiting EPC mobilisation using a VEGF-R2 targeting anti-angiogenic drug, or in Id1/Id3 KO transgenic mice, enhances the anti-tumour activity of the vascular disrupting agent, suggesting that EPCs mediate resistance to the treatment. For this reason it was proposed that EPCs may have a possible role in determining the optimal biological dose of anti-angiogenic drugs (Shaked et al., 2006; Bertolini et al., 2006; Ferrara, 2004; Taylor et al., 2012). However the reasons why EPCs acquire resistant properties contributing to tumour spread are still unknown.

Transplantation of genetically modified bone marrow progenitors may represent a vehicle for the transport of cytotoxic genes, even if the successful translation of gene therapy strategies to tumour treatment is limited due to vector toxicity, poor transduction efficiency and lack of specificity. Another approach is to block the genes involved in the homing of EPCs to tumour vasculature in order to improve anti-angiogenic and anti-tumour effects. In any case EPCs can be genetically engineered *ex vivo* by transduction with retrovirus and lentivirus vectors, which allow long-term transgene expression (Ferrari et al., 2003; Debatin et al., 2008; Jevremovic et al., 2004). The primary advantage of using tumour-targeting EPCs is stable expression of gene targets. Moreover EPCs can be easily expanded and manipulated without showing problems of immunological intolerance. However, other issues are associated with their possible use as an anti-tumour tool. First, only a small number of EPCs are able to be incorporated into tumour vessels. Secondly, the transient resistance of therapeutic EPCs to their cytotoxic effectors results in a strong bystander effect on surrounding tumour cells. Finally, the choice of the most effective cytotoxic effector to be delivered by the EPCs is difficult to determine because of the limited studies available (Debatin et al., 2008).

It has been demonstrated that the delivery of therapeutic agents to tumour cells enhances the drug concentration in cancer tissues, improving their anti-tumour efficacy (Brigger et al., 2002; Ruoslahti et al., 2010; Büll et al., 2015). The concept of targeted drug delivery is attractive because it allows for both high local concentration of the drug and low systemic exposure in patients. In particular, nanoparticles were used as a drug vehicle to target tumour tissues or cells, protecting the drug from premature inactivation during its transport. *In vitro* and *in vivo* experiments were performed to target EPCs for therapeutic cardiac regeneration (Cheng et al., 2014b; Kyrtatos et al., 2009). Nanoparticles have been used as a carrier to target stem cells, including bone marrow–derived endothelial precursors, at a specific location *in vivo* (Kim et al., 2009; van Noort et al., 2009). The availability of specific tumour vascular markers opens a new avenue for delivering therapeutic agents, genes or prodrug enzymes with nanodevices which may reduce pathological angiogenesis (Anderson et al., 2005; Mulder et al., 2005).

Another strategy that can be used to inhibit EPC recruitment is to reduce the pro-angiogenic factors released in the microenvironment mainly by macrophages. Transplantation of genetically modified bone marrow progenitors with lentiviral vectors expressing genes from transcription-regulatory elements of the Tie2/Tek gene significantly reduces the formation of new vessels in tumour sites (De Palma et al., 2003). The Tie2/Tek gene is preferentially expressed in endothelial cells and encodes an angiopoietin receptor tyrosine kinase. The same vector was used also to deliver interferon alpha (INF-α) to tumours and resulted in reduction of new-vessel formation (De Palma et al., 2008). INF-α is a cytokine that shows clinical benefit in the treatment of certain types of cancer. However, because a high dose is required to obtain a good response, this regimen has been phased out due to its high toxicity. The tumour-targeted INF-α delivered by Tie2-expressing monocytes is directly recruited into the tumour site and reduces angiogenesis through down-regulating the expression of proangiogenic factors in tumours. This new anti-cancer therapy may allow for reduction of EPC recruitment without any substantial cytotoxic effects because of its specificity and low-dose requirement.

6. Summary and future work

Bone marrow-derived EPCs may play an important role for tumour progression due to their ability to form new vessels and secrete paracrine factors that direct tumour cells to distant sites. The speculation that EPCs play a crucial role in cancer onset and progression has been confirmed by clinical studies that show the high correlation of this cell population with relapse, disease progression, metastasis and treatment response in human malignancies such as breast, ovarian and non-small-cell lung carcinomas. However, more studies are necessary to improve our knowledge about the signalling pathways involved in tumour angiogenesis associated-metastasis. Elucidating the exact cellular and molecular mechanisms of tumour angiogenesis modulated by the EPCs is crucial not only for determining the role of EPCs in pathological angiogenesis itself but also for better understanding the dynamics that regulate tumour spread. The increased number of bone marrow-derived EPCs is a common feature in various malignancies; hence this minor subpopulation of the mononuclear cell fraction in peripheral blood may represent the common thread between different malignancies, representing a good starting point for the investigation of novel pathways involved in cancer. However, the involvement of epigenetics in all physiological and pathological processes makes this scenario more complicated. In fact, the role of epigenetics has not been completely elucidated and has attracted increasing interest. Perhaps the main challenge in cancer research is to understand the contribution and the link between environment (especially the chronic inflammation state), genetics and epigenetics. In this sense EPCs may represent a turning point.

Some groups are focusing on new strategies to reduce EPC number, and consequently tumour progression, by delivering therapeutic compounds using nanoparticles. These studies are at the pre-clinical stage thus are too early to be translated into clinical practice.

The increase of EPCs in peripheral blood of cancer patients, and their variation in response to therapies, suggests a possible role as biomarkers to predict the state of certain cancers but also to determine the optimal biological dose of anti-angiogenic drugs considering that EPCs are able to mediate resistance to some anti-cancer treatments. However, the standard enumeration procedures or specific parameters of EPCs have not yet been fully established, thus more evidence is needed to verify these observations.

In summary, EPCs seem to represent a novel point for the study of tumour angiogenesis. However, the discovery and characterization of EPCs is very recent, with epigenetics being highly involved in the alteration of EPCs. It is an undeniable fact that further studies are necessary to understand what genetic and epigenetic changes occur in EPCs in response to tumour microenvironment and whether it is possible to translate the emerging discoveries into clinical practice by optimising the isolation and analysis of EPCs.

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