Endothelial Progenitor Cells from Bench to Antitumor Therapy and Diagnostic Imaging

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Abstract

Cancer is the second leading cause of death worldwide after cardiovascular diseases, accounting for an estimated ten million deaths annually. Researchers are making a great effort to identify more efficient therapeutic strategies. To date, genetically modified stem cells are a potential candidate for the development of new antitumor therapies and diagnostic investigation methods.

Among stem cells, endothelial progenitor cells (EPCs), a subpopulation of multipotent hematopoietic stem cells (HSCs), appear promising. In response to specific stimuli, EPCs are fundamental to tumor progression because of their role in vasculogenesis and sprouting angiogenesis. In a healthy adult individual, the process of neoangiogenesis is activated only during wound healing and in the female uterus during ovulation. Therefore, it is reasonable to use them in anti-cancer therapy by taking advantage of their natural tropism to the altered micro-environment. Diverse studies demonstrated that EPCs predominantly home into the tumor mass, and hence, they are useful as a cellular vehicle for site-directed drug targeting to the tumors or for the delivery of imaging probe.

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This chapter explores the underlying molecular mechanisms and the potential application of stem cell therapy in cancer with special reference to EPCs application in targeted gene therapy. How they could be modified, obtained in a significant amount, and administrated to treat cancer has been discussed.

Keywords

Angiogenesis · Cancer therapy · Diagnostic imaging · Endothelial · EPCs · Progenitor cells · Vasculogenesis

Abbreviations

5-FC	5-fluorouracil
Ac-LDL	acetylated low-density lipoprotein
ASCs	adult stem cells
BMMCs	bone marrow mononuclear cells
CD	cytosine deaminase
CEPCs	circulating EPCs
CFU-ECs	colony-forming unit-EC
DR4	death receptor 4
DR5	death receptor 5
ECFCs	endothelial colony-forming cells
ECs	endothelial cells
EC-SPs	endothelial cell-side progenitors
eNOS	nitric oxide synthase
EOCs	endothelial outgrowth cells
EPCs	endothelial progenitor cells
EPO	erythropoietin
ESCs	embryonic stem cells
FBS	fetal bovine serum
FSCs	fetal stem cells
GCV	ganciclovir
GMCSF	Granulocyte-macrophage colony-stimulating factor
GSCs	glioma stem-like cells
HIF-1α	hypoxia-inducible factor 1 alpha
HPCs	hematopoietic progenitor cells
HSCs	hematopoietic stem cells
iPSCs	induced pluripotent stem cells
MAPCs	multipotent adult progenitor cells
MCP-1	monocyte chemotactic protein
MRI	magnetic resonance imaging
MVD	microvessel density
NK	natural kill cells
PARP	poly ADP-ribose polymerase
PBMCs	peripheral blood mononuclear cells

PD-1	programmed death-1
PEI2k	polyethylenimine 2 kDa
PlGF	placental growth factor
PSCs	placenta stem cells
QQc	quality and quantity culture
SCs	stem cells
SDF	stromal cell-derived factor
Sirt1	sirtuin-1
SPECT	single-photon emission computed tomography
SPIO	superparamagnetic iron oxide
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
UEA1	Ulex europaeus agglutinin 1
uPAR	urokinase-type plasminogen activator
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
VESCs	vascular endothelial stem cells
VW-EPCs	vascular wall endothelial progenitor cells
vWF	von Willebrand factor
VW-VSCs	vascular wall-resident vascular stem cells

Introduction

Cancer is a vast group of diseases that share some characteristics. Cancer cells can develop in all tissues/organs of the body, have a high proliferation rate, and can invade the normal surrounding tissue and beyond. Metastasizing is a leading cause of death from cancer (Dillekas et al. 2019). Cancer is the second dominant death source worldwide after cardiovascular diseases, accounting for an estimated ten million deaths annually (Bray et al. 2018). Lung, breast, colorectal, prostate, stomach, liver, esophagus, cervix uteri, thyroid, and bladder cancers are, in order, those with the highest incidence (Bray et al. 2018). As a general trend, patients' survival rate and life quality are improving thanks to early diagnosis, prevention campaigns, and improved standards of care. Despite this, patients' physical and economic efforts and the entire health system make cancer a huge problem and a considerable challenge for researchers.

Different therapeutic designs are distinguished according to the type of cancer and the stage of development. These include surgery, radiation therapy, chemotherapy, immunotherapy, targeted therapy, hormone therapy, stem cell transplant, precision, and personalized medicine (NCI 2020). Surgery for cancer treatment is called curative surgery and is usually applied when the tumor mass is well-confined to a specific body part. Before and/or after resection, the patient could be treated with radiotherapy or chemotherapy. Radiation therapy includes different approaches such as external beam radiation therapy, internal radiation therapy (brachytherapy), oral or systemic radiation therapy, and photodynamic therapy. The operating principle consists of high-energy electromagnetic waves or molecules that create DNA

damage in proliferating tumor cells. Chemotherapy consists of using one or more drugs that act mainly against proliferating cells and thus against cancer cells to prevent or limit their growth and spread. Immunotherapy is a treatment that uses cells or molecules of the immune system, such as use of antibodies or vaccines, or T-lymphocytes, to restore or boost the patient's immune system. Targeted therapy applies drugs designed to "target" specifically cancer cells or cells of the surrounding microenvironment without affecting normal cells, exploiting their unique expression of some genes or proteins. Hormone therapy is a systemic one in which hormones are administrated to destroy cancer that depends on them to grow, like breast and prostate cancers that depend on sex hormones. Stem cell transplant is exploited to replace the patient's bone marrow cells treated with chemo and/or radiation therapy against such cancers as leukemia and lymphoma. Precision and personalized medicine is the newest approach and is based on the patient's genome and epigenome characterization because there is high intra-tumoral heterogeneity. Still, it is only in clinical trials for now.

Among these approaches for cancer treatment and to adopt high-performance methods in terms of improved therapeutic efficacy and fewer undesirable effects, stem cell transplant, alone or in combination with other therapies, could be the right strategy for treatment and the development of new diagnostic investigation methods due to its enhanced target on tumors.

Stem Cells

In all development stages from the embryo to the adult, all organs and tissues possess undifferentiated precursor cells, mitotically active, multipotent, and capable of regenerating mature cells, called stem cells (SCs). They are a reservoir of precursor cells playing a homeostatic role essential for replacing dead or damaged cells due to trauma or diseases (Galli et al. 2003). SCs are highly undifferentiated cells that do not possess morphological, structural, molecular, and antigenic characteristics found in the tissue's differentiated cells to which they belong.

SCs can perpetuate themselves through their ability to self-renew (Weissman et al. 2001). In general, the in vivo self-renewal last for the organism's whole life, but in vitro, it is unlimited under the appropriate experimental conditions. Two types of stem cell divisions are distinguished, symmetric cell divisions and asymmetric ones (Shahriyari and Komarova 2013). In symmetric divisions, the two daughter cells are identical to each other and to the mother cell (expansive symmetric division) or, in the alternative, identical to each other but different from the mother (differentiative symmetric division), called progenitors. In asymmetric division, a stem cell produces one differentiated cell and one stem cell. This system allows the number of stem cells to remain constant at the end of each cell generation. It offers the enormous advantage of increasing or decreasing the number of stem cells within a tissue.

Another critical feature of stem cells is multipotentiality, which is the ability to give rise to a differentiated progeny comprising all types of cells of the residence tissue or, in the case of embryos, to all cells of the adult organism. Stem cells,

according to their potential, are classified as totipotent if they are not specialized and can give rise to a new embryo, such as embryonic cells at the stage of 4–8 cells after 4–5 days from fertilization; pluripotent, if they have the potential to differentiate into all cell types that derive from the three embryonic layers (endoderm, ectoderm, and mesoderm), but they do not have the potential to give rise to an embryo, such as embryonic stem cells (ESCs) at the blastocyst stage with 20–30 cells, after 5–7 days from fertilization; multipotent, if they can differentiate in all cell types of a specific organ or tissue such as hematopoietic stem cells (HSCs); unipotent, if they can give rise to a single cell type such as keratinocytes (Łos et al. 2019).

SCs can also be recruited where they are required to participate in the repair process, thanks to a controlled process called homing, and once they reach the site, they settle there (engraftment). Their well-directed migration is under the control of cytokines gradient and is used to regenerate damaged tissues.

Based on the source, stem cells are classified into ESCs, such as cells isolated from human blastocysts; fetal stem cells (FSCs), such as gonadal cells from abortive fetuses; umbilical cord stem cells, such as cells isolated from cord blood umbilical of newborns; placenta-derived stem cells (PSCs), isolated from the placenta of newborns; adult stem cells (ASCs), isolated from adult tissues such as HSCs; induced pluripotent stem cells (iPSCs), obtained by dedifferentiation of mature cells to embryonic cells by genetic manipulation. iPSCs open new therapeutic opportunities, which are practically the same as those of human embryonic stem cells, but without ethical and scientific concerns.

ASCs, present in small quantities in stem niches of the whole organism, remain quiescent until disease or trauma reactivate them inducing proliferation and differentiation. The niche is a tissue location where a dynamic and specialized microenvironment regulates stem cell biology (proliferation, maintenance, or differentiation). They are present in different organs and tissues: the hematopoietic system (Osawa et al. 1996), brain (Galli et al. 2000; Goritz and Frisen 2012), dermis (Toma et al. 2001), muscle (Qu-Petersen et al. 2002), and liver (Shafritz et al. 2006). Until recently, it was generally thought that ASCs could at most differentiate into all cell types of the tissue they belong to (Price et al. 2007). However, today, it has been observed that, under optimal set of conditions, they can differentiate into other cell types, in addition to those of the original tissue. For example, after bone marrow transplantation enriched with HSCs, they can differentiate in all the three germinal layers' cells (Jackson et al. 2001; Mezey et al. 2000; Orlic et al. 2001; Theise et al. 2000).

Stem cells are applied in regenerative medicine for diseases such as Parkinson's disease (Ourednik et al. 2002), spinal cord damage (Teng et al. 2002), multiple sclerosis (Pluchino et al. 2003), amyotrophic lateral sclerosis (Clement et al. 2003), stroke (Liu et al. 2009), retinal degeneration (Li et al. 2006), Alzheimer's disease (Barnham et al. 2004), myocardial infarction (Jackson et al. 2001), and others. The unique self-renewal and differentiation potential of stem cells are the primary reasons for their use to regenerate damaged organs and correct congenital diseases. However, a major limitation for the therapeutic use of stem cells is the risk of iatrogenic oncogenesis.

The source of the cells for therapy could be the same patients (autologous transplantation) or a donor (allogeneic transplantation). The main attraction is for the immune-privileged autologous stem cells that express the major histocompatibility complex 1 (MHC1), but not MHC2, clusters of differentiation because these can be used in immunocompetent patients, avoiding side effects and with better therapeutic efficacy and significantly improved safety. For instance, in a preclinical study to evaluate EPCs for target gene therapy, it was shown that these cells do not express MHC-I, are resistant to lysis by non-activated natural kill cells (NK), and survive and participate in tumor blood vessel formation after intravenous injection (Wei et al. 2004).

An Overview of Endothelial Progenitor Cells

EPCs are mostly unipotent stem cells capable of differentiating into endothelial cells (Khakoo and Finkel 2005). In vivo, they can differentiate from hemangioblasts, bone marrow multipotent adult progenitor cells (MAPCs), and myeloid/monocytic cells. In vitro, the early and late EPCs are distinguished (reviewed in (George et al. 2011)). Furthermore, to be present as such in bone marrow, peripheral and umbilical cord blood, EPCs can be produced by transdifferentiation of stem cells present in various tissues and organs, under the influence of adequate microenvironments for endothelial differentiation (for extensive EPCs sources, readers can consider the reviewing article (Chopra et al. 2018)).

EPCs express endothelial markers such as CD133, CD31, CD34, CD146, and VEGFR2 and do not express the hematopoietic marker CD45 or mature ECs markers including VEGFR1, VE-cadherin, and Von Willebrand factor (vWF) (George et al. 2011; Medina et al. 2017). $CD34^+/CD133^+/VEGFR2^+$ cells are usually, but not unambiguously considered EPCs (Medina et al. 2017).

EPCs can be studied in two ways, flow cytometry or in vitro culture (Medina et al. 2012). Flow cytometry is used for studying circulating EPCs (CEPCs) in the blood samples where they are quantified as the percentage of mononuclear cells $CD34^+/VEGFR2^+/CD133^+$ (Peichev et al. 2000; Wu et al. 2007). In vitro culture methods are applied to study EPCs derived from peripheral blood mononuclear cells (PBMCs) or by direct flushing of bone marrow mononuclear cells (BMMCs) and expanded using endothelial-specific media. During in vitro culture, two different cell types can be generated, the early and late outgrowth cells being hematopoietic and endothelial, respectively (see Table 1) (Medina et al. 2010). Only the last ones are considered valid EPCs (see Table 2) (Banno and Yoder 2019). The late outgrowth cells, also called endothelial colony-forming cells (ECFCs), originate from $CD45^-/CD133^-/CD34^+$ MNCs, in vitro arise after 7 days, have a highly proliferative polygonal shape, do not differentiate into hematopoietic cells, and produce vascular tube in vitro and in vivo. Moreover, ECFCs can affect neovascularization in vivo, take up acetylated LDL, bind to Ulex europaeus agglutinin 1 (UEA1), and express the surface markers CD31, vWF, CD105, CD146, VE-cadherin, and VEGFR2 (Timmermans et al. 2009; Yoder et al. 2007). Some studies have shown a synergistic

Table 1 EPCs isolated using *in vitro* culture methodologies classification based on a specific phenotype and a biological function

	Name	
In vitro	MACs; early outgrowth EPCs; early EPCs; hematopoietic EPCs; CACs; PACs; CFU-ECs; CFU-HILL; small EPCs; Myeloid EPCs	ECFCs; late outgrowth EPCs; LATE EPCs; non-hematopoietic EPCs; OECs; BOECs' EOCs; large EPCs
From PBMCs or umbilical cord blood appear after	4–10 days	>2 weeks
Achieve peak growth at	2–3 weeks	4–8 weeks
Survive up to	4 weeks	12 weeks
Markers	$CD45^+$ $CD14^+$ $CD31^+$; $CD146^-$ $CD34^-$	$CD31^+$ $CD105^+$ $CD146^+$ VE-$cadherin^+$ vWF^+ $VEGFR2^+$; $CD45^-$ $CD14^-$
Role in new vessel formation	Do not differentiate into ECs but promote angiogenesis through paracrine factors that indirectly augmented proliferation, migration, and the tube forming capability of ECFCs	Became ECs and participate in new blood vessel formation or vascular repair
Secretion/ expression of pro-angiogenic factors	VEGF; IL-8; MMP9	VEGFR2; CXCR-1; MMP2

The table shows the complex EPCs nomenclature and the two leading EPCs population features studied for their pro-angiogenic properties

Abbreviations: *BOECs* blood outgrowth ECs, *CACs* circulating angiogenic cells, *CFU-ECs* colony-forming unit-EC, *CFU-HILL* colony-forming unit HILL EPC, *ECFCs* endothelial colony-forming cells, *EOCs* endothelial outgrowth cells, *MACs* myeloid angiogenic cells, *PACs* pro-angiogenic hematopoietic cells, *PBMCs* peripheral blood mononuclear cells, *OECs* outgrowth ECs

effect of early and late outgrowth cells when used together compared with one of the two cells alone as cell therapy (Pearson 2010; Yoon et al. 2005).

Both early and late EPCs promote angiogenesis. Early EPCs contribute to new vessels formation by secreting a series of growth factors and cytokines, such as VEGF, stromal cell-derived factor-1 (SDF-1), granulocyte colony-stimulating factor (G-CSF), and insulin-like growth factor 1 (IGF-1), which stimulate ECs proliferation and survival, and direct endogenous progenitor cell recruitment into sites of neo-vascularization (Urbich et al. 2005). Furthermore, early-EPCs provide relevant protective effects to themselves and differentiated EPCs from apoptosis under oxidative conditions in an auto- or paracrine manner, recruiting other cells within the peripheral blood (Yang et al. 2010). Late EPCs directly contribute to vasculogenesis by providing structural support via differentiation into mature ECs (Hur et al. 2004). They can also promote angiogenesis by the secretion of numerous cytokines (Moubarik et al. 2011).

Table 2 How to distinguish "bona fide" EPCs?

	MACs	ECFCs	ECs
Potency Assys (capacity to form a vascular network in vitro and in vivo)	Only in conditioned media	Intrinsic tube forming capacity	Intrinsic tube forming capacity
Detailed identity immunophenotype	$CD14^+$	$CD31^+$	$CD31^+$
	$CD31^+$	$CD34^+$	VE-$cadherin^+$
	$CD45^+$	$CD105^+$	$VEGFR1^+$
	$CD34^-$	$CD133^+$	vWF^+
	$CD146^-$	$CD146^+$	$CD34^-$
		VE-$cadherin^+$	$CD133^-$
		$VEGFR2^+$	
		vWF^+	
		$CD14^-$	
		$CD45^-$	
Clonogenicity capacity: (single-cell colony-forming)	Lack	High	Lack
Proliferative capacity	Medium	High	Low

The table shows the tests that must be performed to identify bona fide *EPCs unequivocally. The term EPCs should be restricted to only those cells that display vessel-forming potential, the right immunophenotype, and have high clonogenic potential and proliferation rate*
Abbreviations: *ECFCs* endothelial colony-forming cells, *ECs* endothelial cells, *MACs* myeloid angiogenic cells

Under physiological conditions, EPCs homing-in is aimed to maintain vascular integrity during repair of damaged tissues, restore organ function, and participate in postnatal angiogenesis (Asahara et al. 1997, 1999a; Urbich and Dimmeler 2004). However, EPCS' vasculogenic potential is also exploited by tumors to facilitate their progression (Asahara et al. 1999a; Dong and Ha 2010). As shown in preclinical research, in response to endogenous and exogenous signals, $VEGFR2^+$ EPCs can get mobilized from the bone marrow into the peripheral blood circulation and subsequently home-in to tumor neovascularization sites where they differentiate into ECs, thus contributing to angiogenesis (Nolan et al. 2007; Rafii et al. 2002). Endogenous signals released from tumor cells and their microenvironment induce hypoxia-inducible factor 1-alpha (HIF1-α) overexpression, glucose reduction, and reactive oxygen species increase. These events promote the release of VEGF, SDF-1, monocyte chemotactic protein (MCP-1), and erythropoietin (EPO), which facilitate EPCs homing-in to neovascularization sites (Annese et al. 2019; Dong and Ha 2010; Ribatti 2004). More precisely, this occurs before the angiogenic switch in the avascular tumor phase (Gao et al. 2008). Once recruited, the EPCs can directly participate in new blood vessel formation or can merely release pro-angiogenic factors. The neoangiogenesis is also sustained by co-mobilization of $VEGFR1^+$ hematopoietic progenitor cells (HPCs), which home-in to the tumor-specific pre-metastatic sites and form cellular clusters, the so-called pre-metastatic niche (Kaplan et al. 2005). There is convincing evidence from both preclinical and clinical studies that exogenous signals, such as disruptive vascular agents, chemotherapy,

and surgery, might induce an acute release of EPCs from bone marrow, contributing to tumor growth (Bertolini et al. 2003; Furstenberger et al. 2006; Roodhart et al. 2009; Shaked et al. 2008). Of particular importance is the ability of EPCs to home-in not only into the tumor's vasculature but also into the tumor proper.

Endothelial Progenitor Cells in Neovascularization

The development of EPCs-based therapies to induce or suppress new blood vessel formation necessitates the comprehension of cellular and molecular mechanisms of neovascularization. EPCs have a role in both vasculogenesis and angiogenesis. Physiological vasculogenesis is also known as developmental vasculogenesis because it occurs during embryo development. From hemangioblast, which is a common precursor of hematopoietic and vascular systems, EPCs differentiate in the bone marrow and then extravasate into the peripheral circulation in response to VEGF/VEGFR2 stimuli. From circulation, EPCs follow the stimuli gradient and upon arrival at the site of injury, they differentiate into mature ECs and participate in the ongoing vascular development (Masuda and Asahara 2003). In the adult, these EPCs from the bone marrow could participate in physiological blood vessel formation and pathological one during the early phase of tumor neovascularization.

Vasculogenesis involves the recruitment and participation of circulating cells, and de novo formation of blood vessels from these cells, while angiogenesis results from the proliferation of existing blood vessels. To be more precise, two types of angiogenesis are distinguished as sprouting and non-sprouting angiogenesis. Sprouting angiogenesis occurs when ECs migrate (tip cells) toward the VEGF gradient source and proliferate (stalk cells) to form abluminal sprouts that subsequently fuse and generate new vessels (Risau 1997; Uccelli et al. 2019). On the other hand, non-sprouting angiogenesis, or intussusceptive angiogenesis, occurs in the absence of a gradient, and all ECs respond to VEGF by assuming a stalk phenotype. During intussusception, an already existing vessel splits into two by forming intraluminal endothelial pillars, which fuse longitudinally (Risau 1997; Uccelli et al. 2019). Angiogenesis plays an essential role throughout embryonic development, besides wound healing, tissue ischemia, and tumor vasculature formation during postnatal life. Hence, it is now being exploited as a novel therapeutic target in cancer treatment. During angiogenesis, EPCs can indirectly contribute to tumor vascularization via autocrine/paracrine mechanisms (Asahara et al. 2011).

In addition to the extravasation of EPCs from the bone marrow and homing-in to the site of injury, the neovascularization process is also supported by immature cells present in the vascular wall of various organs. These cells are called vascular wall-resident vascular stem cells (VW-VSCs) that differentiate in smooth muscle cells and ECs (Tamma et al. 2020; Torsney and Xu 2011). The subpopulation called vascular wall EPCs (VW-EPCs) differentiate in ECs and are also known as endothelial cell-side progenitors (EC-SPs) $CD200^{+}/CD157^{+}$ (Ingram et al. 2005; Wabik and Jones 2015). In hypoxia conditions, these cells are under self-renewal and differentiation to stalk cells contributing to long-term ECs proliferation and, thus, angiogenesis (Takakura 2018).

Given the ubiquitous EPCs' role in neovascularization, their concentration in peripheral blood can be a surrogate biomarker indicating vasculogenic/angiogenic tumor activity and therapy efficacy on tumor vasculature as currently done with microvessel density (MVD) and VEGF expression (Bianconi et al. 2020; Nico et al. 2008; Schluter et al. 2018). EPCs concentration is advantageous because it is accurately but noninvasively compared to MVD and VEGF evaluation, but it is disadvantageous because only 0.025% of the PBMCs are EPCs (Peichev et al. 2000). The small amount limits the translation of prosperous findings of EPCs from bench to practical use. EPCs amount is even less if EPCs from VESCs in the preexisting blood vessels are considered. Therefore, for EPCs-based therapy, they should be first expanded ex vivo.

Endothelial Progenitor Cells Applications

EPCs contribute to tissue regeneration processes via neovascularization through paracrine mechanisms or differentiation in mature ECs (Asahara et al. 1999a; Kalka et al. 2000). The freshly isolated autologous PBMCs or BMMCs have been applied to clinical vascular regenerative therapy in patients with peripheral arterial disease, critical limb ischemia, or myocardial infarction (Deutsch et al. 2020; Koshikawa et al. 2006; Kudo et al. 2003; Lara-Hernandez et al. 2010; Li et al. 2016a; Liotta et al. 2018). These researches indicated that cell-based therapy was safe, feasible, and useful.

Potential stem cell applications against cancer have been well-reviewed elsewhere (Chu et al. 2020) are for cell transplantation, post-cancer treatment, vaccine production, therapeutic carriers, or immune cells generator. Clinically, EPCs can be employed in three different manners: (i) for neovascularization; (ii) as target cells in anti-EPCs therapy against tumors; (iii) as biomarkers for disease identification and severity (Chopra et al. 2018).

With the purpose of neovascularization, vascular EPCs can be exploited for their ability to release several angiocrine growth factors, or other bioactive molecules, to maintain and sustain tissues/organs' regeneration, for example, by increasing the releasing of oxygen and nutrients through neoangiogenesis. EPCs present in preexisting blood vessels or recruited from bone marrow could be used in vascular regeneration therapy in many diseases like revascularization of ischemic tissues after heart infarction (Huang et al. 2013; Moubarik et al. 2011; Steinle et al. 2018). EPCs could be applied to non-angiogenic and angiogenic tumors to induce blood vessel formation, which will be a direct access route of the drug on the tumor cells, or induce blood vessel normalization, which will alleviate hypoxia and pro-tumor microenvironment, respectively (Collet et al. 2016).

As potential therapeutic carriers, a Trojan horse, EPCs combined with targeted antiangiogenic drugs for cancer treatment act as a delivery vehicle that protects the therapeutic agents from rapid biological degradation, reduce systemic side effects, and increase local therapeutic levels due to the intrinsic tumor-targeting effect. Recent advances in cellular engineering have led to stem cell-based vector development to serve as a vehicle for angiogenesis inhibitors or genes directly into the tumor

endothelium (Janic and Arbab 2010; Nakamura et al. 2004). These novel approaches are useful in oncology to selectively destroy cancer cells, leaving healthy cells unaffected thus alleviating the side effect of cancer therapy (Chong et al. 2016; Ruggeri et al. 2018). For instance, in Sprague-Dawley rats, EPCs isolated from PBMCs were genetically modified to induce a stable expression of antiangiogenic endostatin, reducing VEGF expression. These genetically modified EPCs were successfully tested to suppress retinal vascular leakage and could be advanced for clinical assessment because endostatin overexpression may serve as a potential therapeutic agent (Ai et al. 2018).

Antitumor treatments' efficacy is usually evaluated by imaging techniques such as X-ray, computed tomography, magnetic resonance imaging (MRI), and ultrasound. EPCs can be used for diagnosis, prognostic prediction, and follow-up. EPCs can be labeled for CD133 for tracking their in vivo fate after injection by MRI for diagnosis and follow-up. As biomarkers of tumor development and/or progression, several studies have demonstrated clinical correlations between CEPCs concentration and tumor stage (Nowak et al. 2010; Ramcharan et al. 2013; Yu et al. 2013), tumor size (Richter-Ehrenstein et al. 2007; Su et al. 2010), VEGF serum concentration (Rafat et al. 2010; Yang et al. 2012), and MVD (Li et al. 2018a; Maeda et al. 2012). During hematological malignancies' comparison with solid tumors, many studies have demonstrated a close association between EPCs and disease activity, so much so that circulating EPCs are useful diagnostic, therapeutic, and prognostic biomarker (Ge et al. 2015; Ruggeri et al. 2018).

The EPC-based therapies are much better known for cardiovascular diseases compared to oncological ones. EPCs were proposed to induce angiogenesis in ischemia (Li et al. 2018b; Zheng et al. 2014), for post-injury vascular endothelial regeneration (Abd El Aziz et al. 2015; Guo et al. 2017), and ex vivo tissue engineering (Sales et al. 2010). However, the enthusiasm for the possible applications of EPCs in clinical therapy is severely limited by the lack of in-depth characterization and understanding of early and late outgrowth EPCs.

Endothelial Progenitor Cells Sources, Ex Vivo Culturing, and Implantation

Mouse, monkey or human ESCs, fetal liver, human umbilical cord blood, bone marrow, and peripheral blood might be used as the potential EPCs sources (Debatin et al. 2008; Zakrzewski et al. 2019). The use of stem/progenitor cells from embryos is advantageous and ideal because they can show unlimited and undifferentiated proliferation and evade immunological rejection as they do not express MHC-I. Still, there are ethical considerations and risk of malignant transformation that restrict their progress to the clinical setting (Wei et al. 2004; Werbowetski-Ogilvie et al. 2009). EPCs derived from the fetal liver can be easily isolated and cultured; however, the clinical applicability of these cells is limited by the challenges of creating fetal liver tissue banks and host immune incompatibility (Cherqui et al. 2006).

Stem/progenitor cells present in umbilical cord blood have a higher proliferative capacity, readily available, and easy to isolate than adult bone marrow-derived cells.

However, umbilical cord donation has yet to achieve widespread acceptance, besides the chance of immunologic graft-versus-host disease in the recipients (Ingram et al. 2005; Murohara 2001; Murohara et al. 2000; Qin et al. 2017).

Also, multipotent adult progenitor cells isolated from postnatal bone marrow have extensive proliferation potential ex vivo and can differentiate into mesodermal lineage cells as EPCs (Reyes et al. 2002). Thus, they can be effectively applied in autologous therapy, thanks to potentially low-level immune recognition and destruction of these cells by the host immune system.

More recently, EPCs have been isolated from human adult somatic cells, that is, fibroblasts, through transdifferentiation into iPSCs (Purwanti et al. 2014; Taura et al. 2009). Nevertheless, like ESCs, EPCs iPSCs-derived will need an in-depth characterization to exclude tumorigenic potential. An easily accessible EPCs' source is either peripheral blood or bone marrow. Circulating autologous EPCs could be isolated from these sources using markers like CD34, CD133, or VEGFR2 (Asahara et al. 1997; Shi et al. 1998). Circulating EPCs and bone marrow-derived EPCs are among the least complicated sources to use, but the main obstacle in their use in regenerative medicine is low quality and quantity, and immune recognition (Asahara et al. 2011; Sukmawati and Tanaka 2015). Chemotactic molecules as VEGF, placental growth factor (PlGF), granulocyte-macrophage colony-stimulating factor (GMCSF), or statins are used to treat patients/donors to increase EPCs number (Asahara et al. 1999b; Dimmeler et al. 2001) (for schematic EPCs application as therapy see Fig. 1).

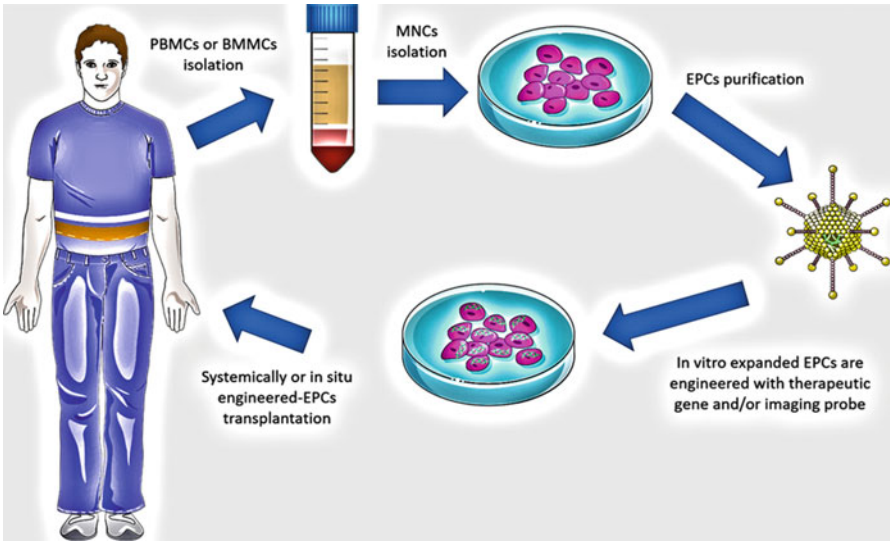

Fig. 1 Schematic representation of EPCs as cellular vehicles. EPCs derived from PBMCs or BMMCs are expanded and transduced *in vitro* to express pro-drugs or tratted for the expression of imaging probe can be systematically or in-situ replanted in the patients.

EPCs, after isolation from PBMCs or BMMCs, are expanded in vitro. There are several optimized protocols available for ex vivo culture and expansion of EPCs. However, all of these protocols require that EPCs are plated on extracellular matrix proteins coated dishes and maintained in endothelial basal medium with added supplements and growth factors (Au et al. 2008; Kawamoto et al. 2001; Lu et al. 2014). Concerning supplements, EPCs' expansion is expensive and time-intensive due to high concentrations and frequent supplementation of growth factors because of their short half-life at physiological temperatures (Khalil et al. 2020). Moreover, supplements, such as fetal bovine serum (FBS), could be unsafe for clinical application due to their animal origin, prone to batch-to-batch variations, xenoimmunization, and possible contamination of mycoplasma, viruses, endotoxins, and prions (Dessels et al. 2016). The development of optimal protocols to expand EPCs without growth factors is a promising approach to simplifying clinical translation. Interestingly, polyphenols benefit EPCs number and functional activity (Di Pietro et al. 2020; Huang et al. 2010). For example, an attractive agent to expand EPCs is the natural flavonoid quercetin. It increases the number and functional activity of EPCs and protects them against serious glucose-induced damage by inducing Sirtuin-1 (Sirt1)-dependent endothelial nitric oxide synthase (eNOS) upregulation (Zhao et al. 2014). To avoid the use of animal serum, several laboratories have developed novel serum-free expansion methods enriched with optimal cytokine and growth factor combinations (Hagiwara et al. 2018; Kado et al. 2018; Masuda et al. 2012). These methods are known as the quality and quantity culture (QQc) system and ensures optimizing EPCs-based therapy by augmenting their qualitative and quantitative vasculogenic properties and providing measurable regenerative capacity (Sukmawati and Tanaka 2015).

To isolate and expand EPCs, it is imperative to consider that EPCs are decreased in number and functional activity related to age and cardiovascular risk factors (Huang et al. 2014; Kaur et al. 2018). Therefore, isolation and application of EPCs from patients with these backgrounds have a high chance of receiving EPCs with low therapeutic effect.

Before administration, expanded EPCs are characterized for their morphology, surface markers expression by flow cytometric analyses, eNOS levels, and Ac-LDL uptake/fluorescein isothiocyanate (FITC)-lectin binding actives. Moreover, their routine functional characterization also involves assessment of angiogenic potential by in vitro tube formation assay or in vivo by chick chorioallantoic membrane (CAM) assay (Kukumberg et al. 2020; Merckx et al. 2020; Qin et al. 2018; Song et al. 2010). Furthermore, recent studies advise performing isolated cells' efficacy and safety, for example, by transplantation in a nonhuman primate model (Qin et al. 2018).

Once collected sufficient number in vitro, EPCs can be conditioned to enhance their functionality for more efficient functionality and therapeutic benefits. Several studies about the revascularization of ischemic tissues have employed various growth factors or recombinant proteins or genes using nano- or microparticles to improve tissues' revascularization and upregulate pro-angiogenic proteins (Bhise et al. 2011; Simon-Yarza et al. 2012). Recombinant proteins are costly, and it is not

easy to maintain adequate protein levels at the target region due to their relatively short half-lives (Gupta et al. 2009). Gene therapy with viral and nonviral delivery system was applied as an alternative strategy to express the desired pro-angiogenic proteins, and it has shown to be promising.

EPCs can be genetically manipulated by stable transduction using retrovirus or lentivirus-based vectors encoding for the gene/s of interest, allowing a long-term transgene expression. Besides their high transduction efficiency, their use is convenient when EPCs are used as a vehicle due to their susceptibility to gene transduction protocols. However, since viral vectors are usually replication-defective, there will be a physiological clearance reduction of EPCs vehicle following administration. This reduction can be advantageous if a temporary rather than stable presence of the cells is the goal of cell therapy. EPCs can also be modified with non-integrating viral vectors such as adenovirus or herpes simplex virus, or plasmid vector, inducing short-term effects. Moreover, using a non-integrating method, the EPCs can be modified with synthetic mRNAs, which can express exogenous proteins without the hazard of insertional mutagenesis since the delivered mRNAs remain in the cytoplasm for translation without passing into the nucleus (Sahin et al. 2014; Steinle et al. 2018). The synthetic mRNA transfection leads to the transient production of exogenous proteins of interest in the cells, and subsequently undergoes natural degradation thus leaving no traces of the delivered mRNA.

After isolation, modification, and characterization, EPCs can be implanted in patients. Generally, stem cells could be introduced in different ways, such as intravenous, intramuscular, intra-articular, and intrathecal (Saeedi et al. 2019). Intravenous is the safest and most straightforward method to deliver the EPCs throughout the body. This way is a preferred route administration as it is simple and feasible, does not require general anesthesia, and allows administration of repeated doses at different times (Haider et al. 2017). The transplanted EPCs mainly home-in to a tumor site due to attraction to the tumor vasculature by its angiogenic drive, but the efficiency is not 100%. In effect, most cells intravenously inject end up in the non-target sites, including lungs, liver, and spleen (Leibacher and Henschler 2016; Varma et al. 2013b). Several factors could explain the lack of efficiency, including tumor microenvironment composition variability, vascular network size, or angiogenic stimulus power (Li et al. 2011; Wang et al. 2016). Moreover, after intravenous administration, EPCs have to compete with the endogenous EPCs for incorporation into the target organ such as a tumor. This necessitates the suppression of endogenous EPCs. One of the strategies for improving EPCs tumor homing is to deliver these cells as an adjuvant to chemotherapy or radiation therapy because this can increase the migration and incorporation of EPCs into the tumor (Shaked et al. 2008). Furthermore, to improve EPCs delivery and homing-in capacity, they might be directly injected into the arterial circulation or infused at multiple time-points (Dudek 2010; Lin et al. 2020).

To successfully translate EPCs into cell-based therapy for routine patient application, it is critical to develop a technique to monitor transplanted cells' in vivo biodistribution after delivery. Among the in vivo cell-tracking and cell-fate determining techniques, magnetic resonance imaging (MRI) is one of the most powerful

one because of its satisfactory resolution (Aicher et al. 2003; Hu et al. 2012; Wang et al. 2003). For MRI, the cells are labeled with MRI contrast agents that are not efficiently loaded into cells to avoid cytotoxicity (Crabbe et al. 2010; Hu et al. 2012). An alternative label is the paramagnetic agent IronQ, a complex of iron and quercetin, added to cell culture (Kantapan et al. 2017). It is not only traceable by MRI but also serve as cell proliferation inducer (Kantapan et al. 2017).

Endothelial Progenitor Cells in Preclinical Cancer Studies

Searching for clinical trials involving EPCs in cancer therapy, it is possible to find only five studies, all providing information regarding the characterization and quantification of CEPCs using biomarkers (ClinicalTrials.gov Identifier: NCT00393341; NCT00753610; NCT00325871; NCT00826683; NCT00067067). These studies are based on complete evidence about the emerging role of CEPCs in tumor angiogenesis as surrogate markers of antiangiogenic therapies efficacy.

It is widely known that neovascularization is a crucial cancer hallmark that facilitates cancer cells proliferation and progression. Blood vessels deliver oxygen and nutrients to cancer cells allowing them to grow further 2 mm in diameter. When tumor mass is over, in response to hypoxia and microenvironment signals, cancer cells overexpress molecules that promote vasculogenesis, angiogenesis, and evasion. As discussed earlier, resident ECs and EPCs, which line all blood vessels or are present in the peripheral circulation respectively, or are recruited from bone marrow migrate toward an angiogenic cue, proliferate and form new vessels. EPCs' involvement in tumor vasculogenesis, contributing to the development of vascular network or vascular mural cells, and their homing-in to the site of tumor angiogenesis means that they have access to distant and, in most cases, undetectable micrometastases are the reasons behind the use of these cells in cancer therapy (Rajantie et al. 2004; Reyes et al. 2002).

In an experimental mouse orthotopic hepatoma model developed using tumor liver cell line HepG2, Zhu et al. (2012) demonstrated that intravenous tail-vein injection of BMMCs-derived EPCs preferentially migrated into the site of tumor development (liver) compared to the other organs. They also demonstrated that EPCs migrated to the tumor site in response to the cytokines (VEGFR, HIF-1α, SDF1) cues secreted by the tumor cells. On the contrary, some researchers have reported failure of EPCs' chemotaxis in all kinds of tumors in response to the chemical cues emanating from the tumor cells (Annabi et al. 2004; De Palma et al. 2005; Larrivee et al. 2005; Lyden et al. 2001; Purhonen et al. 2008).

The orientated homing of EPCs in hepatomas as in other tumors enhances their possible clinical applications as delivery vehicles for suicide gene therapy, anti-angiogenesis gene therapy, or tumor suppressor gene therapy. An effective EPCs-based strategy in cancer therapy is to genetically manipulate EPCs with the genes encoding for the enzymes that metabolize pro-drugs into pharmacologically active anticancer drug derivatives that would kill the surrounding cancer cells based on a spectator effect (bystander effect) (Freeman et al. 1993; Zweiri and Christmas 2020).

The death of the donor EPCs and their ineffectiveness against rapidly growing or large tumor limit these suicide gene therapies after drug activation (Wei et al. 2007). During the last few years, the effectiveness of an exciting tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) anticancer activity has been reported (Lim et al. 2015; Yuan et al. 2018). TRAIL initiates the pro-apoptotic pathway by selectively binding with its death receptors-4 and -5 (DR4, DR5), while sparing the healthy cells unaffected (Forster et al. 2013; Kichev et al. 2014). Deng et al. (2018) engineered EPCs (isolated from PBMCs of neonatal Sprague-Dawley rats) with a lentivirus encoding for TRAIL for glioma treatment. TRAIL has a short half-life and also fail to cross through the blood-brain barrier and (Guo et al. 2011; Holoch and Griffith 2009). Thus, EPCs-based TRAIL gene delivery has overcome these problems (Choi et al. 2016; Redjal et al. 2015; Wang et al. 2014). TRAIL-EPCs migrate to glioma cells SHG44 in the transwell assay and induce glioma cell apoptosis in a co-culture in vitro system by increasing the cleaved caspase-3 and -8 levels and poly ADP-ribose polymerase (PARP) (Deng et al. 2018). To solve EPCs ineffectiveness due to their small number, an indirect strategy is to target tumor vasculature cells, which are critical for tumor growth and survival, instead of the whole enormous tumor cell mass. For instance, Dudek et al. (2007) have shown that genetically engineered EPCs over-expressing the antiangiogenic molecules endostatin significantly decreased tumor vascularization and growth after tail vein injection into NOD-SCID vein mice with subcutaneously implanted Lewis lung carcinoma cells. Laurenzana et al. (2014) developed a personalized therapy against melanoma using autologous MMP12-engineered EPCs to treat both tumor cells and tumor vasculature.

MMP12 is a metalloelastase with a bivalent role: protective if expressed by macrophages and non-protective if expressed by tumor cells (Houghton et al. 2006; Margheri et al. 2011; Martin and Matrisian 2007). MMP12 application as an anticancer strategy is based on MMP12's enzyme activity to cleave urokinase-type plasminogen activator (uPAR). The full-length isoform acts as a potent endothelial activator responsible for tumor progression. uPAR can be expressed by both endothelial and tumor cells (Andolfo et al. 2002). Laurenzana et al. (2014) demonstrated that EPCs transfected with a lentivirus encoding for MMP12 are recruited into melanoma mass under CXCR4/SDF1 system stimuli after intravenous delivery in experimental settings. Moreover, in vitro and in vivo, it was shown that MMP12-engineered EPCs reduced melanoma progression, intra-tumoral angiogenesis, and lung metastasis in old CD-1 nude mice, degrading uPAR on tumor cells and ECs (Laurenzana et al. 2014). Noteworthy, these ex vivo MMP12-engineered EPCs lost the capacity to perform capillary morphogenesis in vitro and, at the same time, acquired the antitumor and antiangiogenetic activity. Thus they seem to show no side effects in vivo (default pro-angiogenic role) (Duda et al. 2000). EPCs-based therapy using genetically transduced cells was also applied in a preclinical study for nasopharyngeal carcinoma. Wang et al. (2018) demonstrated that EPCs genetically modified with a lentiviral encoding for the metastatic gene suppressor KAI1/CD82 successfully inhibited lung metastasis in a nude mice bearing human nasopharyngeal carcinoma xenografts. However, there was little evidence regarding their potential to suppress the tumor cell graft.

A third EPCs-based strategy in cancer gene therapy is to accentuate the host immune system against cancer. For instance, Ojeifo et al. (2001) engineered EPCs to express IL-2 to stimulate natural killer and cytotoxic T cells in a syngeneic mouse model of melanoma lung metastases. They demonstrated that multiple intravenous injections abrogated the tumor metastases and prolonged animal survival. Muta et al. (2003) manipulated EPCs with a retrovirus vector carrying IL-12, showing that, in vivo, this gene therapy selectively delivers the protein to the tumor site in a xenograft rat model of breast cancer where its overexpression induced natural killer and cytotoxic T cells.

To overcome the controversies associated with the ESCs from human embryos, recently, iPSCs are considered the primary source of autologous or allogeneic pluripotent stem cells. They were explored as a source of human EPCs suitable as a delivery system of immune-stimulatory molecules to inhibit cancer. Purwanti et al. (2014) obtained $CD133^{+}/CD34^{+}$ EPCs from human iPSCs. They demonstrated that the cells expressed EPC-specific markers (i.e., CD31. VEGFR, cadherin) did not express hematopoietic cell markers (i.e., CD45), exhibited tubulogenesis in vitro, showed tumor tropism in an orthotropic lung metastasis mouse model for breast cancer, and did not enhance tumor growth and metastasis. Moreover, when these iPSCs-EPCs, engineered with a baculovirus encoding for the immune co-stimulatory molecule CD40 (with a pivotal role in the T-cell activation), were systemically injected in breast cancer-bearing mice, the animals showed prolonged survival (Purwanti et al. 2014). Noteworthy, in this study, an insect baculovirus was used instead of the conventional animal viral vectors. Insect virus bypasses the risk of virus replication and infection in the human host cells, and there is no host immune response (Bessis et al. 2004; Strauss et al. 2007). However, they are not adapted for long-term transgene expression.

A combination of suicide gene-targeting therapy with an antiangiogenic molecule was established in a human HepG2 liver cancer preclinical model to improve patients' treatment outcomes. Zhang et al. (2020) developed a gene therapy protocol with cytosine deaminase (CD) and endostatin gene transfected in EPCs obtained from fresh heart blood of adult BALB/c nude mice. Cytosine deaminase is one of the most widely investigated suicide gene/pro-drug that converts the nontoxic antifungal agent 5-fluorocytosine into the toxic chemotherapeutic agent 5-fluorouracil (5-FC) (Lawrence et al. 1998). The abovementioned preclinical model showed a total tumor volume reduction by MRI, angiogenesis inhibition visualized by VEGF- and CD31-positive immunostaining, decreased ECs, and increased tumor cell apoptosis assessed by TUNEL assay in mice transfected with CD/endostatin-EPCs plus 5-FC (intraperitoneally injected) compared to control treatment group (Zhang et al. 2020). CD/endostatin synergistic action could be translated in to clinical trials to target the hepatomas site via vein grafting.

Neoangiogenesis is mostly proven via CD31/VEGF immunohistochemistry, but this method is inadequate because it requires experimental animals to be sacrificed or human biopsies taken for immunohistological studies renders follow-up is impossible. Recently, studies are focused on advanced, noninvasive, and real-time molecular imaging methods as tracking strategies to monitor transplanted EPCs-based drug

vectors for antitumor therapy (Arbab et al. 2006). EPCs can be applied for noninvasive MRI investigation, as demonstrated by Chen et al. (2014a). They have approximately 100% of human PBMCs-derived EPCs efficiently labeled with N-alkyl–polyethylenimine 2 kDa (PEI2k)-stabilized superparamagnetic iron oxide (SPIO) nanoparticles. Moreover, functional assay outputs such as proliferation, migration, and tubulogenesis rates and incorporation into tumor neovasculature in vivo results have shown that these magnetic-labeled EPCs have the same activity as unlabeled ones. Once labeled, EPCs were intravenously or subcutaneously injected in a lung carcinoma xenograft model and were effectively detected by seven-tesla micro-MRI at the tumor site. The results showed excellent biocompatibility and magnetic resonance sensitivity even at a small alkyl-PEI2k/SPIO concentration than other contrast agents (Chen et al. 2014a). An EPC-based theranostic method has also been also proposed using the abovementioned MMP12-engineered EPCs radiolabeled with ^{111}In 8-oxyquinoline (oxine) for all the tumors displaying uPAR-dependent cancer progression (Laurenzana et al. 2014).

In glioma, the herpes simplex virus TK (HSV-TK)/ganciclovir (GCV) gene therapy is a suicide gene therapy widely used in both experimental and clinical trials thanks to its potent bystander effect (Zhang et al. 2010). A combination of HSV-TK suicide gene therapy with real-time molecular imaging has been reported for glioblastoma. Varma et al. (2013a) employed human cord blood-derived EPCs as a delivery vehicle for replication-competent adenovirus AD5 carrying both suicide genes, yeast CD (yCD) and mutant HSV-TK mutTK (SR39), and reporter gene, human sodium iodide symporter (hNIS) for I-131 (radioiodine) for diagnostic MRI imaging and single-photon emission computed tomography (SPECT). Their results indicated that AD5-yCD-mutTK-EPCs reached the glioma mass upon intra-tumor injection. Furthermore, double staining experiments demonstrated that both EPCs ($hNIS^+/vWf^+$) and tumor cells ($hNIS^+/EGFR^+$) expressed the transgenes thanks to the transfected EPCs' ability to deliver the vectors in the surrounding tumor cells (Varma et al. 2013a). Noteworthy, this study exploited intra-tumor injection instead of the prevalent systemic injection. The intra-tumor injection is advantageous to alleviate the virus's entry into circulation and curtail side effects (Lohr et al. 2001). Moreover, a replication-deficient virus is used as a transgenes delivery system to improve tumor cell death by their self-replication properties and infectivity of the surrounding cells in the vicinity (Barton et al. 2011; Barton et al. 2003). The reporter gene system with hNIS also overcame the short monitoring time (~7 days) with In-111Oxine labeling. hNISm allows repeated detection of the injected cells for extended periods (Barton et al. 2003; Varma et al. 2013a).

EPCs have also been proposed as the best vehicle to deliver therapeutic genes and imaging probe targeting glioma stem-like cells (GSCs) (Chen et al. 2014b). Glioma is a vascular-rich tumor with high resistance to antiangiogenic therapy because tumor cells can pass from the vascular phase of growth to the nonvascular one and vice versa. The mechanisms by which glioma achieve neovascularization are vascular co-option, angiogenesis, vasculogenesis, vascular mimicry, and GSCs-ECs transdifferentiation. GSCs-ECs transdifferentiation is implicated in the resistance against anti-VEGF therapy which currently in practice (Baisiwala et al. 2019;

Yan et al. 2017). Using in situ C6 glioma rat model, Chen et al. (2014b) showed that exogenous spleen-derived EPCs labeled with USPIO (ultra-small SPIO) integrate into the vessels containing glioma-derived ECs without inducing any promoting effect of GSCs transdifferentiation.

Despite these promising results, in vivo MRI with iron-nanoparticles presents some inconveniences: (i) the signal is lost over time due to the contrast agent biodegradation or dilution following cell division; (ii) time of EPC migration to the tumor site depends on multiple factors, such as tumor location and size, and chemotaxis factors expression levels; (iii) it is challenging to monitor real-time EPCs' migration into blood circulation; and (iv) imaging devices may lead to different results due to its sensitivity and resolution.

To enhance in vitro expanded EPCs translation from preclinical studies to clinical trials, the in vivo safety issues should be addressed because adverse effects and responses caused by EPCs therapy have been reported, such as collapse, sepsis, breast cancer development, and even death (Granton et al. 2015). In this context, Lee et al. (2019) proposed EPCs transplantation in dogs as a possible safety test of deleterious effects that should be conducted before EPCs application in human clinical trials. The choice of dogs lies in their physiological similarity with humans. They performed physical and laboratory examinations of human EPCs isolated from healthy donor PBMCs and transplanted intravenously into dogs. This in vivo safety assessment could be useful to test the minimal number of EPCs for transplantation because a high number is associated with pulmonary emboli or infarctions and affect immune responses (Beggs et al. 2006; Grigg et al. 1996; Prockop and Olson 2007).

Systemic delivery of EPCs gene therapy to primary tumors and metastases is the most attractive feature of using EPCs. Nevertheless, it remains to understand what factors permit EPCs persistence during hypoxia, migration, and proliferation to angiogenic sites, and if they are detained within the blood vessel wall or migrate further outside, and if they participate to vessel maturation.

Side Effects and Potential Risks of EPCs Cell Therapy

Given their advantages, EPCs are anticipated to play a pivotal role in cancer theranostics, for both therapy and biomarkers in future. However, various issues relevant to their use must be resolved before routine clinical use. New optimized stem cell differentiation protocols and animal models must be explored to better understand the molecular events involved in EPCs generation and differentiation. How to isolate and unequivocally identify the phenotype and functionality of EPCs remains problematic. The standardization of cell culture conditions, doses, and administration schedules will make it easier to understand different studies' results to interpret their data for future applications (Morales-Cruz et al. 2019).

EPCs are rare in both peripheral blood (0.01%) and bone marrow (0.05%). This necessitates their in vitro expansion to get them in large number for in vivo use. However, in vitro culture may alter their immunologic characteristics and tumorigenic potential. For example, during in vitro expansion, EPCs are exposed to

exogenous culture conditions different from physiological niches' microenvironment wherein stem cell proliferation and differentiation are under maintained under strict control. Consequently, EPCs could change their genome and phenotype that could render them tumorigenic thus contributing to tumor initiation. On the same note, sub-culturing will reduce stemness at every passage. This means that the development of new EPCs isolation methods is required to improve their yield as well as quality.

EPCs have a natural tropism for the sites of vascular injury. To avoid systemically adverse effects when used as delivery vehicles, advances in nanotechnology and tissue engineering are required to improve EPCs' homing-in and incorporation to the site of interest. When EPCs are employed to target drugs or genes to tumor cells, they could cause drug toxicity or drug resistance. For instance, after systemic injection, a small amount of them will reach the tumor site because most of them will be trapped in the lung, liver, or lymph nodes, causing therapeutic ineffectiveness and drug resistance (Brooks et al. 2018).

Another side effect could be a viral infection when viral carriers are used to genetically engineer EPCs (Goswami et al. 2019). Viral vectors currently employed in patient's treatment are classified as non-integrating or stable host-genome integrating vectors. The former include adenovirus and adenoassociated virus vectors and are primarily used for in vivo gene delivery in patients. Adenovirus can accommodate a large cDNA but are highly immunogenic. In contrast, adeno-associated viruses can only accommodate a smaller cDNA and are less immunogenic but retained for a longer time in the non-dividing cells. The last are retroviruses and lentiviruses. Both can harbor small cDNAs such as adeno-associated viruses, but unlike these, they allow for the prolonged-expression of the therapeutic gene, although there is a risk of insertional mutagenesis.

The evaluation of EPCs source for transplantation is essential. Allogeneic or autologous (via iPSCs technology) stem cell transplantation may provoke severe host immune responses or autoimmunity, respectively (Li et al. 2016b). Hematologic and lymphoid cancers are commonly treated by allogeneic HSC transplantation, but often patients incurred in Graft-versus-Host Disease (GVHD), acute or chronic, due to the induction of a complex immunological reaction of the donor's immunocompetent cells toward the recipient's tissues and organs. Different studies confirmed significantly improved outcomes, with a reduced incidence of chronic GVHD, but not acute one, after umbilical cord blood transplantation compared to allogeneic hematopoietic stem cell transplantation (Chen et al. 2017; Narimatsu et al. 2008).

It is necessary to consider the modulation of the host microenvironment as well. Cells and molecules of the microenvironment during hypoxia or increased inflammation have adverse effects on EPCs survival. For example, given the complexity and immunosuppressive properties of the tumor microenvironment, stem cell transplant combined with other therapies, such as immune checkpoint inhibitors, may better eliminate cancer and its recurrence. For example, Hu et al. engineered the surface of HSCs with the checkpoint inhibitor programmed death-1 (PD-1) antibodies-decorated platelets for the treatment of recurrent leukemia in mice (Hu et al. 2018).

A general recommendation is to pay attention when choosing EPCs as a therapy in oncology: it must be considered that mobilization and integration in tumor blood vessels depend on tumor type, stage, and treatment (Farnsworth et al. 2014). For example, cell therapies hold much more promise for treating diseases in which tissue can be ablated, such as bone marrow or skin cancers that can be easily removed with drugs or surgically, respectively. These procedures favor transplanted cells' engraftment because they will not have to compete with diseased resident cells. In more morphological complex tissues such as the brain, where massive ablation of diseased tissue is impossible, engraftment of transplanted cells is lower, and consequently, therapeutic efficacy is reduced.

Conclusion and Future Perspective

The chapter explores the underlying molecular mechanisms and potential applications of EPCs in cancer therapy. The chapter also discusses the protocols to obtain EPCs in a significant amount and their modification, administrated, besides the possible undesired effects and potential risks for cancer patients.

Despite that cancer is one of the leading public health problems, there are still no adequate and exhaustive therapeutic and diagnostic protocols available due to the incomplete knowledge of cancer cell biology. Among the various approaches for cancer theranostics, manipulated-stem cell transplant, alone or as an adjuvant for other therapies, could be a new strategy to treat cancer patients. Stem cells reside in almost all organs and tissues in the body, with the potential for self-renewal, migration, and differentiation that justifies their use in antitumor therapy. Therefore, studying stem cells for tissue engineering and theranostic resolutions is exciting. The existing results concerning stem cell therapy for cancer are highly encouraging. ESCs and iPSCs are the most powerful ones, but the diversity in their applications is still limited due to the possible risks related to viral vectors and ethics issues. EPCs are one of the autologous stem cell types for human use as they lack MHC-I expression, resistant to NK-mediated cytolysis, and primarily involved in blood vessel formation besides ease of availability and isolated and expanded ex vivo/in vivo, efficiently transduced to carry a therapeutic payload and home-in to the tumor and its vasculature. Hence, they are excellent cellular vehicles for systemic and local cancer therapy in general and angiogenic cancer in particular, as they primarily depend on blood vessels for growth and metastasis. It would be interesting to interfere with tumor vascularization by restoring a balance between pro- and antiangiogenic signaling and ensure direct access to drug delivery at the tumor site (Collet et al. 2016). Therefore, EPCs can be manipulated to selectively deliver the therapeutic molecules to the cancer cells while sparing the healthy cells. Given their biocompatibility and sensitivity when labeled, EPCs may serve as near-ideal vasculature tracker for diagnostic imaging.

Currently, only CEPCs are in clinical trials as surrogate biomarkers of antiangiogenic therapy. However, to validate the diagnostic value of CEPCs, the selection criteria of both cancer patients and healthy controls should be stricter due to the

Table 3 Summary of EPCs' advantages and disadvantages

EPCs versus other stem/progenitor cells in cancer therapy		
	Advantages	Disadvantages
Isolation	Less complicated	Low amount
Ex vivo expansion	Can generate enough cells for therapy	Massive tests must be performed to identify bona fide EPCs
Transduction	Efficient	
Tropism	Home to tumor proper and tumor vasculature	Only a minority of systemically administrated EPCs incorporate into tumor vessels
Drug	Protect the drug from inactivation	Systemically exposure
Immunological tolerance	Autologous EPCs not affected	

involvement of numerous confounding factors, that is, background cardiovascular diseases, diabetes mellitus, and lifestyles, which include smoking status and physical exercise, among others (Mayr et al. 2011).

Despite success in preclinical experimental animal models and enormous possibilities yet to be explored, the cell availability in small number, low-quality preparations, poor retention, low survival rate, and engraftment after transplantation still hamper EPC's routine clinical application (Sukmawati and Tanaka 2015; Terrovitis et al. 2010). Besides, there are no unique identifying markers for EPCs, and functional characterization of the rare putative EPC population-based on FACS phenotypes is challenging to realize for a large dataset. Hence, a consensus on the exact characterization and biology of EPCs is required to create a standardized, generally accepted methodology to develop the use of EPCs in clinical settings for regenerative approaches (Sabbah et al. 2019). Another drawback is the optimization of culturing protocols containing media without animals-derived supplements. Moreover, the establishment of stem cell-based anticancer therapies is slowed down by the lack of adequate financial support, the existence of ethical and political issues, and the easy authorization of new therapeutic protocols for which the efficacy has not been adequately tested. The current gap between public expectancy and actual progress of stem cell-based therapies in the clinical threatens regenerative medicine's social license to operate (Cossu et al. 2018). A possible step forward is to develop a combinatorial approach on several fronts (tumor vasculature, tumor cell tumor microenvironment, immune system) to achieve a better outcome. In conclution, EPCs translation from bench to antitumor therapy and diagnostic imaging depends on a more in-depth assessment (Table 3).

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