Chapter 7
Endothelial Progenitor Cell Therapy in Stroke

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Contents

7.1 Stroke 127
7.2 Endothelial Progenitor Cells (EPCs) 128
7.2.1 Discovery of EPCs 128
7.2.2 Source of EPCs 128
7.2.3 Methods for the Isolation and Culturing of EPCs 129
7.2.4 Classification of EPCs 131
7.3 Therapeutic Potential of EPCs for Stroke 132
7.3.1 A Biomarker of Diseases 132
7.3.2 Protection of Blood Brain Barrier (BBB) 132
7.3.3 Promotion of Neovascularization After Stroke 135
7.3.4 Factors Influence EPCs In Vivo 135
7.4 Signaling in Regulating EPC Functions 137
7.5 Action Mechanism of EPCs 137
7.5.1 Cell Replacement 137
7.5.2 Enhanced Trophic/Regenerative Support for Endogenous Repair Processes 138
7.6 Transplantation of EPCs in Ischemic Stroke Animals 139
7.6.1 Transplantation Routes for EPCs 139
7.6.2 Modification of EPCs 140
7.7 Pros and Cons in the Application of EPCs in Clinical Trials 146
7.8 Problems Need to be Clarified for the Treatment of Patients 146
7.8.1 Evaluation of Clinical Safety 146
7.8.2 Identifying Acceptable Patients for EPC Transplantation 147
7.8.3 Time, Dose, Route and Type of EPCs for Transplantation 148
7.8.4 Bio-Distribution and Persistence of EPCs 149
Conclusions 149
References 150

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Abstract Stroke is a major cause of death globally, which induces irreversible neuronal and endothelial cell death. Endothelial progenitor cell (EPC) based therapeutics result in neovascularization and the improvement of vascular perfusion, which benefits clinical stroke patients. Although EPC transplantation in experimental stroke models shows functional improvement, EPC therapy in clinical stroke patients continues to face an arduous task. In this chapter, we give a brief introduction of EPCs including the source of EPCs, methods of isolation and identification of EPCs, the therapeutic potential for stroke, and signaling in modulating EPC function. Furthermore, we summarize the molecular mechanisms of EPC action after transplantation either through differentiating into mature endothelial cells to replace damaged cells or by enhancing trophic/regenerative support for endogenous repair processes. We discuss the routes of transplantation and the modifying methods for EPC safety and efficacy *in vivo*. Finally, we discuss the pros and cons for the application of EPCs for transplantation in clinical patients. Though EPC-based therapy is a potential treatment for stroke and holds promise for vascular regeneration, this field needs more study to uncover and resolve unsolved problems.

Abbreviations

acLDL	Acetylated low density lipoprotein
ACE2	Angiotensin-converting enzyme 2
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
BM	Bone marrow
CACs	Circulating angiogenic cells
CFU-Hil	Colony forming unit-Hill
ECFC	Endothelial colony forming cell
ECs	Endothelial cells
EGF	Epidermal growth factor
eNOS	Endothelial NO synthase
EPCs	Endothelial progenitor cells
EPO	Erythropoietin
FACS	Fluorescence activated cell
G-CSF	Granulocyte colony-stimulating factor
GDNF	Glial cell line-derived neurotrophic factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HGF	Hepatocyte growth factor
HIF−Iα	Hypoxia-inducible factor−1α
HMGB−1	High-mobility group box 1
HSCs	Hematopoietic stem cells
ICAM−1	Intercellular adhesion molecule 1
IGF−1	Insulin-like growth factor−1
IL−8	Interleukin−8
MCP−1	Monocyte chemoattractant protein−1

MMP−9	Matrix metalloproteinase 9
MNCs	Mononuclear cells
MRI	Magnetic resonance imaging
NPCs	Neural progenitor cells
PB	Periblood blood
PDGF	Platelet-derived growth factor
PlGF	Placental growth factor
SDF−1α	Stromal derived factor−1α
TGF-b2	Transforming growth factor b2
tPA	Recombinant tissue plasminogen activator
UCB	Umbilical cord blood
UEA−E	Ulex europaeus agglutinin 1
VEGF	Vascular endothelial growth factor

7.1 Stroke

Stroke is a multifactorial disease, and ischemic and hemorrhagic stroke are the leading causes of death globally (Hassan and Markus 2000; Rubattu et al. 2000). A variety of risk factors have been illustrated to relate with stroke incidence including cerebrovascular diseases, aging, smoking, hypertension, diabetes, hypercholesterolemia, and lack of exercise. (Hankey 2006; Allen and Bayraktutan 2008; Flynn et al. 2008; Karam et al. 2008). The brain is very vulnerable to ischemic insult because it is sensitive to a lack of oxygen and glucose. Neurological dysfunction usually occurs within minutes after stroke onset. However, the deterioration of the brain may continue in the following minutes, hours or even days.

Ischemic stroke (> 70 % of strokes) is the most common type of stroke in clinical stroke patients. After ischemic stroke onset, a process of pathophysiological events are triggered, including energy failure, loss of cell ion homeostasis, the release of excitatory amino acid and reactive oxygen species, increase of intracellular calcium, disruption of the blood-brain barrier (BBB), activation of glial cells, and the infiltration of leukocytes (Bliss et al. 2007; Moskowitz et al. 2010). These interrelated and coordinated events result in ischemic cell necrosis, which exhibits non-selective damage of all cells including neurons, astrocytes, oliogdendrocytes, microglia and endothelial cells (Broughton et al. 2009). The size and location of these infarcts are determinants of the long-term functional deficits (Sims and Muyderman 2010). The ischemic penumbra area represents the region in which there is a chance for recovery via post-stroke therapy (Ginsberg 1997).

The only effective treatment for ischemic stroke patients is to administer recombinant tissue plasminogen activator (tPA). However, very few patients are lucky enough to receive tPA treatment because tPA has a very narrow time window (<4.5 h). Stem cell therapy has been proposed as a potential treatment for ischemic stroke in recent years, especially after putative progenitor endothelial cells have

been isolated from bone marrow (BM) and identified as CD34 positive (Asahara et al. 1997). This kind of cells is named endothelial progenitor cells (EPCs) and is capable of contributing to the formation of new vessels by differentiating into mature endothelial cells (ECs) or supporting/promoting the endogenous repair process. EPCs can also serve as a marker during stroke occurrence and prognosis (Chu et al. 2008), and preclinical studies have shown EPC transplantation improves functional recovery by promoting neurogenesis and angiogenesis or provide trophic/protective factors through paracrine effects. Several clinical studies are currently investigating the safety and efficacy of EPC transplantation. EPC transplantation in stroke represents a promising therapeutic approach, although it is still in its infancy.

7.2 Endothelial Progenitor Cells (EPCs)

7.2.1 Discovery of EPCs

EPCs are BM mononuclear cells (MNCs), which were first isolated from peripheral blood (PB) by Asahara in 1997 (Asahara et al. 1997). This novel technique opened a new field for the treatment of vascular disease. Increasing evidence showed that EPCs could be mobilized to the PB after ischemic stroke and restore the damaged vessels via vasculogenesis (Asahara et al. 1999; Takahashi et al. 1999; Kalka et al. 2000a; Shintani et al. 2001; Murayama et al. 2002; Asahara and Kawamoto 2004; Zhan et al. 2013). Considering these cells' lack of unique markers, and that they share similar surface antigens with some hematopoietic lineages and mature ECs, such as CD31/KDR (VEGFR-2)/CD34/VE-cadherin/E-selectin (Rafii 2000; Khakoo and Finkel 2005), it is difficult to precisely define EPCs. However, it has been generally accepted that EPCs exist in circulating blood and possess angiogenic capability and the potential to differentiate into ECs, which contribute to the process of vasculogenesis and the maintenance of homeostasis in vascular ECs (Asahara et al. 1997; Shi et al. 1998; Asahara et al. 1999; Lin et al. 2000; Rafii 2000; Cesari et al. 2012). EPCs may be mixed with the circulating ECs in peripheral circulation, which may partially differentiate into mature ECs. EPCs play a more important role in promoting postnatal vasculogenesis compared with circulating ECs (Kalka et al. 2000a). Therefore, EPCs and circulating ECs are two different cells.

7.2.2 Source of EPCs

EPCs can be divided into two types based on their origin: hematopoietic and non-hematopoietic EPCs. Hematopoietic EPCs originating from BM are considered a subtype of hematopoietic stem cells (HSCs). Non-hematopoietic cells could be isolated from PB, umbilical cord blood (UCB), and tissue samples (Asahara et al. 2011). Although the origin of non-hematopoietic cells is unclear, this type of cell is likely derived from organ blood vessels and tissue stem cells (Alev et al. 2011).

In addition, increased studies have reported additional sources of non-hematopoietic cells. For example, the myogenic-EPCs in the interstitial spaces of skeletal muscle contribute to skeletal muscle growth (Tamaki et al. 2002); EPCs could also exist in the boundary between smooth muscle and the adventitial layer of human vascular walls (Zengin et al. 2006). Other sources of EPCs include the liver and intestine (Aicher et al. 2007), dental pulp-derived iPS cells (Yoo et al. 2013), the kidney (Sirker et al. 2009) and adipose tissue (Planat-Benard et al. 2004). Therefore, it is plausible that EPCs could be found in other sources, further study is needed to investigate the mysterious origin of EPCs.

7.2.3 Methods for the Isolation and Culturing of EPCs

Actually, it is a challenge, and controversial work, to isolate and identify EPCs from the PB, because of these cells' lack of unique and specific surface markers. Currently three methods are mainly used to isolate EPCs from the PB. The first and perhaps simplest method is to collect low density MNCs via density barrier centrifugation, and then plate these cells on fibronectin coated dishes with culture medium containing a variety of growth factors and fetal serum. After 4–5 days, remove non-adherent cells (Asahara et al. 2000; Vasa et al. 2001; Tepper et al. 2002). The remaining adherent cells present the early EPCs with spindle shape (Fig. 7.1). The second method is based on cell surface antigens, using a technology known as fluorescence activated cell (FACS) analysis to distinguish EPCs from other cells in PB (Yoder 2009; Kirton and Xu 2010; Basile and Yoder 2014). Although there are no specific antigens to isolate and identify EPCs, some have been accepted as fundamental elements representing the EPC phenotype, which are CD34, CD133 (AC133), and KDR (VEGFR−2) (Peichev et al. 2000). Subsequently, different combinations of these antigens have been used to isolate EPCs from PB, UCB and fetal liver (Timmermans et al. 2009). However, recent studies provide opposing evidence that cells expressing that the three antigens mentioned above should not represent EPCs, but stand for hematopoietic progenitors, because no observed vessel structure formed *in vivo* (Timmermans et al. 2007). Other surface antigens have also been used to identify EPCs, such as CXCR4, CD31, CD144, CD105, CD106, CD117, and CD45 (Basile and Yoder 2014). However, all of these surface antigens, including CD34, CD133 and KDR, do not only emerge on EPCs, but are also expressed on other cells, for example HSCs (Hirschi et al. 2008; Alaiti et al. 2010; Fadini et al. 2012; Yoder 2012), leading to unpersuasive results when isolating and identifying EPCs. Therefore, novel specific markers need to be found to identify true EPCs. The last method includes two colony-forming assays *in vitro*, which are based on cluster formation. One is called colony forming unit-Hill (CFU-Hil) assay. Briefly, the MNCs isolated from the PB are plated on the fibronectin coated dishes for 48 h, and the non-adherent cells are collected to culture again, clusters occur ring after 4–9 days, which are named CFU-Hil EPCs (Hill et al. 2003). These cells have similar characteristics to early EPCs (Fadini et al. 2012), express endothelial and hematopoietic cell markers, and fail to form vessels *in vivo*. The other method

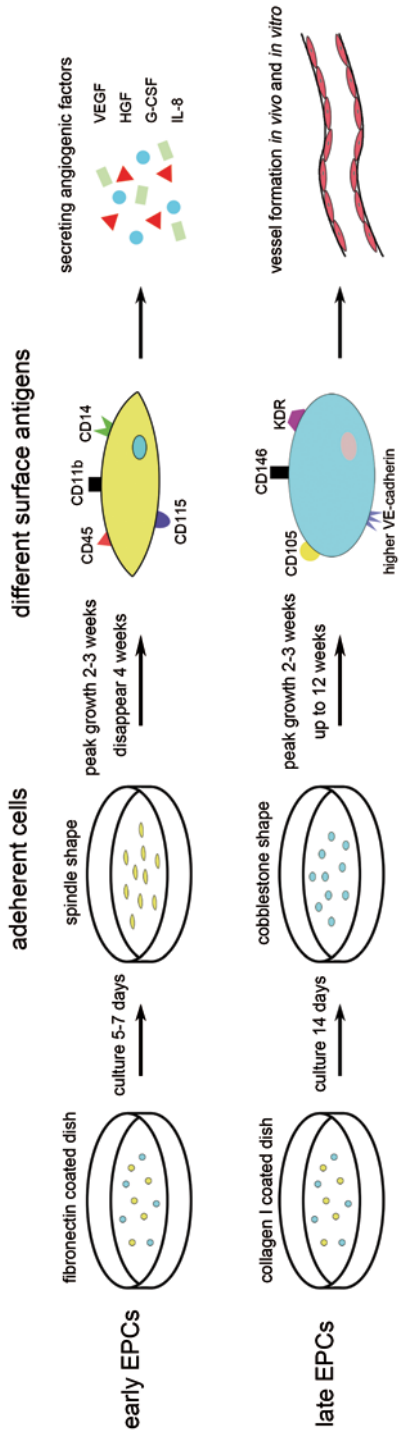

Fig. 7.1 An overview of isolation methods and characteristics of early and late EPCs

Table 7.1 Characteristics of early and late EPCs

	Early EPCs	Late EPCs	Ref
Source	PB	BM, PB/CB, vessel well	(Ingram et al. 2004; Kirton and Xu 2010)
Culture time	5–7 days	>14 days	(Ahrens et al. 2011; Zhao et al. 2013)
Morphology	Spindle	Cobblestone	(Hur et al. 2004)
Survival fate	Peak growth 2–3 weeks, disappear 4 weeks	Peak growth 2–3 weeks, up to 12 weeks	(Zhao et al. 2013)
In vitro	Low proliferative ability, no tube-like structure formation	High proliferative ability, capillary-like formation	(Hur et al. 2004)
In vivo	No vessel formation	Vessel formation	(Hur et al. 2004)
Function	Cannot regenerate a damaged endothelium	Physically contribute to vascular regeneration	(Hur et al. 2004; Yoder et al. 2007; Kirton and Xu 2010)
Neuro-vasculogenesis	Release angiogenic cytokines	Differentiate into endothelial cells	(Rehman et al. 2003; Hur et al. 2004)
Same surface antigens	CD34/KDR (VEGFR−2)/CD31/CD114/vWF	CD34/KDR (VEGFR−2)/CD31/CD114/vWF	(Kirton and Xu 2010)
Different surface antigens	CD45/CD14/CD11b/CD115	CD105/CD146, higher VE-cadherin/KDR	(Hur et al. 2004; Ingram et al. 2004; Kirton and Xu 2010; Fadini et al. 2012)

BM bone marrow, *PB* peripheral blood, *CB* cord blood

is endothelial colony forming cells (ECFCs) assay. Plate the isolated MNCs on collagen I coated dishes, and adherent cells form colonies 2–3 weeks later. These cells are named ECFCs, which are known as late EPCs (Ingram et al. 2004; Kirton and Xu 2010). ECFCs express antigens like primary ECs, have a huge potential to form colonies, and are able to form vessels *in vivo* and *in vitro* (Yoder 2009) (Fig. 7.1).

7.2.4 Classification of EPCs

According to their culture characteristics and functions, circulation EPCs can be classified into two different populations: early EPCs, which are also called circulating angiogenic cells (CACs), and late EPCs, which are also known as ECFCs (Hur et al. 2004) (Table 7.1). The early EPCs emerge 5–7 days after isolation of MNCs from the PB and disappear at 4 weeks. They have spindle shape, can be stained with Ulex europaeus agglutinin 1 (UEA−1) and take up acetylated low-density lipoprotein (acLDL) (Hur et al. 2004; Hirschi et al. 2008). They express EC markers and keep hematopoietic antigen expression (Kirton and Xu 2010). Early EPCs cannot

form vessels *in vivo*, but contribute to angiogenesis by secreting angiogenic cytokines (Gehling et al. 2000; Lin et al. 2000; Vaughan and O'Brien 2012). Late EPCs form a monolayer of cobblestone shaped cells 2–4 weeks after plating, have huge potential to proliferate, and can be maintained for up to 12 weeks. Similarly, these cells can also be stained with UEA−1, take up acLDL and express the same markers as early EPCs, such as CD34/KDR (VEGFR−2)/CD31, but they lack the expression of antigens like CD14, CD133, CD45 and CD115 (Hur et al. 2004; Ingram et al. 2004; Kirton and Xu 2010). More importantly, late EPCs are able to form vessels *in vitro* and *in vivo* (Lin et al. 2000; Grant et al. 2002). Late EPCs are thought to be the true EPCs and show greater sensitivity to vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and placental growth factor (PlGF) (Bompais et al. 2004; Pasquier 2010). Therefore, these two types of EPCs have different morphologies, proliferative abilities and survival rates but both of them display vasculogenic capacity *in vivo* (Hur et al. 2004) (Fig. 7.1).

7.3 Therapeutic Potential of EPCs for Stroke

7.3.1 A Biomarker of Diseases

There is no doubt that EPCs exist in adult PB (Asahara et al. 1997), promote vascular repair after ischemia, and attenuate the progression of arteriosclerosis (Medina et al. 2012). In the past years, a lot of studies have demonstrated that the number and functional stage of circulation EPCs are associated with arteriosclerosis, hypertension, diabetes, and metabolic syndrome (Vasa et al. 2001; Hill et al. 2003; Werner et al. 2005; Liao et al. 2010; Mandraffino et al. 2011; Devaraj and Jialal 2012; Flammer et al. 2012). Based on this evidence, levels of circulation EPCs can be used as novel biomarkers. More importantly, the levels of EPCs also have a close relationship with ischemic stroke, studies have shown that lower levels of circulation EPCs indicate poor outcomes among ischemic stroke patients (Ghani et al. 2005; Sobrino et al. 2007; Chu et al. 2008; Yip et al. 2008; Tsai et al. 2014) (Table 7.2).

7.3.2 Protection of Blood Brain Barrier (BBB)

As we all know, the BBB is comprised of brain microvascular ECs, basement membrane, astrocytes and pericytes, all of these parts are now called the neurovascular unit (Wong et al. 2013). The integrity of the BBB plays an important role in maintaining the homeostasis of the brain. Once destroyed, the balance of the brain's microenvironment is disrupted, leading to a series of pathological processes, including the swelling of endothelial cells, an increase in vascular permeability, inflammatory cell infiltration and tissue edema. As mentioned above, EPCs have the potential

Table 7.2 EPCs as a marker for diseases or good outcomes

Diseases	No. patient	EPC phenotype	EPC number	Marker for	Ref
IS	30	$CD34^+/KDR^+$	↓	Increase burden of carotid atherosclerosis	(Lau et al. 2007)
IS	48	CFU-EC	↑	Good functional outcome; reduced infarct growth	(Sobrino et al. 2007)
Stroke	75-acute 45-chronic	CFU	↓	Endothelial dysfunction or repair in acute stroke	(Chu et al. 2008)
Acute IS	138	$CD31^+/CD34^+$ $CD62E^+/CD34^+$ $KDR^+/CD34^+$	↑in IS ↓in severe neurological impairment	↓EPC: severe neurological impairment and adverse clinical outcomes	(Yip et al. 2008)
CM	214	$CD34^+/VEGFR\text{-}2^+CD34^+/CD133^+$	↓	A pathophysiologic feature of CM	(Gyan et al. 2009)
Acute IS	100	$CD133^+/KDR^+$	↓	Mobilization of EPCs in response to stroke stress	(Zhou et al. 2009)
Acute IS	17	$CD34^+/CD133^+/VEGFR\text{-}2^+$	↓	Larger lesion volumes; acute phase stroke severity	(Bogoslovsky et al. 2010)
IS	42	$CD133^+/CD34^+/KDR^+/CD45^-$	↑	Enhanced angiogenic function in the subacute phase of stroke	(Navarro-Sobrino et al. 2010)
IS	17	$CD133^+/CD34^+$	↓	Tissue injury and stroke severity in early ischemia	(Bogoslovsky et al. 2011a)
CA	27	$CD133^+/CD34^+$	↓	Contributes to the pathophysiological process of aneurysm formation	(Wei et al. 2011a)
Ruptured CA	14	$CD133^+/CD34^+$	↑	Prognostic marker for the outcomes of ruptured CA	(Wei et al. 2011b)
Hypertension	32	$(CD31^+/CD34^+/KDR^+/CD45^-$	↓	Relates to radiological cerebral small vessel disease manifestations	(Rouhl et al. 2012)

Table 7.2 (continued)

Diseases	No. patient	EPC phenotype	EPC number	Marker for	Ref
IAS	108	$CD34^+/CD133^+/VEGFR\text{-}2^+$	↑	Independent markers of IAS	(Liu et al. 2013c)
IS	146	$CD34^+/CD133^+/VEGFR\text{-}2^+$	↑at day 7	A better functional outcome	(Marti-Fabregas et al. 2013)
Acute IS	65	$CD133^+/CD34^+$; $KDR^+/CD34^+$	↓	Poor 6-month outcome in patients with AIS	(Tsai et al. 2014)

CFU-EC early outgrowth colony-forming unit-endothelial cell, *IS* ischemic stroke, *CA* cerebral aneurysm, *CM* cerebral malaria, *IAS* intracranial arterial stenosis

to differentiate into ECs and promote vascular repair (Ponio et al. 2014), and to support the integrity and function of the BBB (Kaneko et al. 2012). However, how EPCs beneficially influence the BBB is still a mystery. Therefore, more work is needed to elucidate the protective mechanism of EPCs on the BBB after stroke.

7.3.3 Promotion of Neovascularization After Stroke

It has been widely accepted that neovascularization after stroke is essential and critical for tissue repair and neurological function recovery. Animal and human studies have proved that EPCs participate in neovascularization (Zhang et al. 2002; Fan et al. 2010; Paczkowska et al. 2013), mainly via two approaches: by directly differentiating into ECs and incorporating into the damaged vessels, which is called vasculogenesis; and by indirectly promoting migration and proliferation of pre-existing ECs, which is called angiogenesis, through releasing a variety of angiogenic cytokines (Masuda and Asahara 2003; Tepper et al. 2005; Urbich et al. 2005; Chen et al. 2013b). In addition, these cytokines also enhance EC and neuron survival, and recruit endogenous progenitor cells (Chen et al. 2013b). Because of the unique characteristic and advantage of angiogenic ability, EPCs may be an important agent for the treatment of stroke.

7.3.4 Factors Influence EPCs In Vivo

In the past decade, it had been demonstrated that tissue ischemia and exogenous cytokines could mobilize endogenous circulating EPCs and thereby contribute to neovascularization (Asahara et al. 1999; Takahashi et al. 1999). Subsequently, more and more studies have proven that the levels and functional stages of EPCs are correlated with many diseases and are considered as a biomarker (Table 7.2). Moreover, transplantation of EPCs as a therapeutic strategy is beneficial to the hindlimb and cerebral ischemia (Kalka et al. 2000a; Fan et al. 2010; Moubarik et al. 2011). Recently, several studies showed that a variety of factors could influence the number and function of circulating EPCs *in vivo*. For example, statin treatment for 4 days may increase circulating EPCs levels in acute ischemic stroke patients, probably by nitric oxide (NO)-related mechanisms (Sobrino et al. 2012a). VEGF and stromal derived factor−1α (SDF−1α) are independent factors for the increment of circulating EPCs (Sobrino et al. 2012b). In addition, factors like homocysteine, haptoglobin 1–1, citicoline, cilostazol, systolic blood pressure, total cholesterol, erythropoietin (EPO), high-mobility group box 1 (HMGB−1), and matrix metalloproteinase (MMP−9) are also proven to influence the number of circulating EPCs in humans and animals (Table 7.3). The therapeutic effects of transplantation of EPCs for stroke can be improved by modulating these factors (Morancho et al. 2013).

Table 7.3 Factors that influence EPCs *in vivo*

Factors	EPC source	Route and time for factors of administration	EPC phenotype	Influence	Patients or animals	Ref
Homocysteine	PB	—	$CD34^+/CD31^+$	↑EPCs apoptosis	? patients with hyperhomocysteinemia	(Alam et al. 2009)
Haptoglobin 1-1	PB	—	$CD34^+/KDR^+$	↓EPCs number	42 patients with lacunar stroke	(Rouhl et al. 2009)
BP and total cholesterol	PB	—	$CD133^+/KDR^+$	↓EPCs number	? patients with acute stroke	(Zhou et al. 2009)
SDF-1	PB	—	$CD34^+/CD133^+/VEGF\text{-}R2^+$	↑EPCs number	17 patients with acute IS	(Bogoslovsky et al. 2011b)
Citicoline	PB	Oral citicoline (2000 mg/day)	CFU-EC	↑EPCs number	48 patients with IS	(Sobrino et al. 2011)
Cilostazol	PB	200 mg daily	$CD34^+/CD133^+$	↑EPCs number	20 diabetic patients with leukoaraiosis or asymptomatic old cerebral infarction	(Ueno et al. 2011)
EPO	PB	200 U/kg 1 month after surgery	$CD34^+/CD133^+$	↑EPCs number	rat with CA	(Xu et al. 2011)
EPO	PB	5000 IU Each time, subcutaneously	$CD31^+/CD34^+$, $CD62E^+/CD34^+$, $KDR^+/CD34^+$	↑EPCs number	167 patients with acute IS	(Yip et al. 2011)
HMGB-1	Peri-infarct in brain	—	$CD34^+/Flk1^+$	↑EPCs number	mice with 45 min MCAO	(Hayakawa et al. 2012)
Statins	PB	20 mg atorvastatin/day	CFU-EC	↑EPCs number	48 patients with acute IS	(Sobrino et al. 2012a)
VEGF and SDF-1α	PB	—	CFU-EC	↑EPCs number	48 patients with IS	(Sobrino et al. 2012b)
MMP-9	Spleens	—	$vWF^+/KDR^+/CD133^+$	Delay EPCs release	Animals with MCAO	(Morancho et al. 2013)

BP blood pressure, *PB* peripheral blood, *CFU-EC* Foutgrowth colony forming unit-endothelial cell, *HMGB−M* high-mobility group box 1, *EPO* erythropoietin, *MMP−M* matrix metalloproteinase 9, *IS* ischemic stroke, *CA* cerebral aneurysm, *MCAO* middle cerebral artery occlusion, — no administration, *?* not mentioned

7.4 Signaling in Regulating EPC Functions

Studies are investigating a variety of factors that influence EPC proliferation, migration and maturation (Table 7.3). Additional researchers are trying to discover the signaling pathways activated by these factors to influence EPCs. Early EPCs secrete a large number of factors, including VEGF, brain-derived neurotrophic factor (BDNF), bFGF, insulin-like growth factor 1 (IGF−1), and interleukin−8 (IL−8). (He et al. 2005; Moubarik et al. 2011; Rosell et al. 2013), which are pro-angiogenic factors that increase endothelial proliferation, tube formation, migration and MMP secretion in ECs to enhance the invasiveness of EPCs (Carmeliet 2003; Li et al. 2003). MMP−9 is essential for ischemia-induced neovascularization, which modulates the neovascularization of EPCs by increasing the release of cytokines (Huang et al. 2009; Morancho et al. 2013). Integrin-linked kinase is upregulated in ECs and associated with increased intercellular adhesion molecule 1 (ICAM−1) and SDF−1 under hypoxic stress, which recruits EPCs to ischemic tissue (Lee et al. 2006). CD18 and its ligand ICAM−1 also play an essential role in mediating EPC recruitment in infracted hearts (Wu et al. 2006). Activated AKT signals promoted the expression of ICAM−1 on ECs and closely associated with EPC entrapment, which might be important in regulating the process of neovascularization through enhancing EPC migration and trans-endothelial migration (Yoon et al. 2006; Hur et al. 2007).

IL−10 increases EPC survival and mobilization through the activation of STAT3/VEGF signaling cascades (Krishnamurthy et al. 2011). SDF−1 released from the ischemic tissue form a concentration gradient to promote EPC homing through interaction with its receptor CXCR4 (Fan et al. 2010). Deltalike−4 gene modified EPCs show enhanced functional neovascularization in ischemic tissue due to the activation of Notch/Hey1/mTOR/p70S6K signaling pathways (Huang et al. 2013a). Wnt1 is a pro-angiogenic molecule and enhances EPC function in a hepatocyte growth factor (HGF)-dependent manner (Gherghe et al. 2011). HMGB1 secreted by astrocytes after ischemic stroke increases EPC homing involved in neurovascular remodeling and functional recovery (Hayakawa et al. 2012). Other factors have also been reported to influence EPC functions, such as E-selectin, estrogen and β2-adrenergic receptor (Oh et al. 2007; Tan et al. 2012; Galasso et al. 2013). Fully understanding the mechanisms underlying EPC function will help improve the safety and efficiency of EPC transplantation.

7.5 Action Mechanism of EPCs

7.5.1 Cell Replacement

EPCs derived from BM or other tissues have an intrinsic capacity for differentiating into ECs (Asahara et al. 1997; Beltrami et al. 2003; Planat-Benard et al. 2004; Chen et al. 2008; Chen et al. 2012; Nih et al. 2012; Iskander et al. 2013;

Pellegrini et al. 2013). The injured ECs in the brain can be replaced by transplanted EPCs. Granulocyte colony-stimulating factor (G-CSF) mobilizes circulating EPCs to engage 39% of the total luminal length of the neoendothelium (Takamiya et al. 2006). LacZ-transduced $CD34^+$EPC transplantation leads to about 60% reendothelialization of balloon-injured rabbit carotid arteries costained with CD31 as early as 4 days after transplantation and this increases to about 70% at 30 days after transplantation (Griese et al. 2003b). Fluorescence-labeled EPCs are found in the neointima and costaining with vWF is found after 4 weeks in the injured carotid artery of balloon injured New Zealand white rabbits (Hu et al. 2013). Hence, cell replacement is one of the mechanisms of vascular repair by progenitor cells. BM derived EPCs contribute to the microvascular structure of the choroid plexus by differentiating into ECs during cerebral ischemia in adult mice (Zhang et al. 2002). 14 days after the transplantation of EPCs in the cerebral ischemia rabbit model, a decrease in the number of apoptotic cells and an increase in the microvessel density in the ischemic boundary area has been witnessed, and most of EPCs capable of binding to UEA−1 lectin are incorporated into capillaries (Chen et al. 2008).

However, the extent of incorporation of BM derived cells in cerebral vessels after stroke has varied in previous studies (Hess et al. 2002; Zhang et al. 2002; Machein et al. 2003; Chen et al. 2008; Moubarik et al. 2011). Whereas positive vessels had an average of 34% endothelial marker expressing BM derived cells (Hess et al. 2002; Zhang et al. 2002), others could not detect endothelial marker expressing cells (Machein et al. 2003; Moubarik et al. 2011).

7.5.2 Enhanced Trophic/Regenerative Support for Endogenous Repair Processes

Neovascularization is not solely the result of the incorporation of EPCs in newly formed vessels; the release of trophic factors in a paracrine manner may also influence neovascularization. Cultured PB MNCs secrete high levels of VEGF, HGF, G-CSF and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Rehman et al. 2003). More and more researchers are paying close attention to the trophic effects of EPCs. *In vitro*, early EPCs cultivated from different sources have shown marked expression and the release of angiogenic cytokines including G-CSF, GM-CSF, VEGF, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), FGF, HGF, IL−8, transforming growth factor β2 (TGF-β2), IGF−1 and etc (He et al. 2004; Hur et al. 2004; Yoon et al. 2005). The release of these growth factors in turn may influence the classical process of angiogenesis, particularly the proliferation and migration as well as the survival of mature ECs (Folkman 1995; Urbich and Dimmeler 2004). EPCs can also exert a strong mitogenic effect on mature ECs and enhance the angiogenic capacity of outgrowth of ECs via secretion of IL−8 with/without VEGF (He et al. 2005; Yoon et al. 2005).

In cerebral arteries, the paracrine effect of EPCs promotes vasoprotection by increasing prostacyclin production and the intracellular concentration of cAMP

(Santhanam et al. 2007). EPCs from stroke patients present higher levels of pro-angiogenic factors at early stages, which decrease in mature ECs when they become more similar to mature microvascular ECs (Navarro-Sobrino et al. 2013). 24 h after the administration of EPCs expressing GFP, they are found to express endothelial NO-synthase (eNOS) and distribute in the brain parenchyma and around the endothelial layer of pial arteries in the ischemic lesions (Ohta et al. 2006). EPC transplantation can also induce humoral effects, which are sustained by host tissues, decrease apoptosis and augment proliferation of cells. Transplantation of EPCs enhances the mobilization of endogenous EPCs and HSCs mainly by upregulation of humoral VEGF, FGF−2, IGF, HGF, angiopoietin−1 and SDF−1 (Cho et al. 2007).

Studies have shown that vascular niche can support neurogenesis in the subventricular zone and the dentate gyrus by secreting growth factors associated with neurogenesis, such as VEGF or BDNF (Leventhal et al. 1999; Palmer et al. 2000). In an experimental stroke study, neovascularization related to neurogenesis, and also to the migration of neural progenitor cells (NPCs), along the newly formed vessels (Thored et al. 2007). Thus, administered EPCs may enhance the proliferation of endogenous NPCs in the brain (Rouhl et al. 2008). EPCs injected 24 h after MCAO were found in the injured area and improved functional recovery, which was linked to a reduction in ischemia-induced apoptosis and a stimulation of ischemia-induced angiogenesis and neurogenesis (Moubarik et al. 2011). Transplantation of BM-derived EPCs exerts potent neuroprotective functions against cerebral ischemia/reperfusion injury in rats, and the protective effects may be associated with decreased expression of Bax, caspase−3 and p67phox and the increasing expression of Bcl−2 and manganese superoxide dismutase (MnSOD), which promotes anti-oxidative and anti-apoptotic properties (Qiu et al. 2013).

7.6 Transplantation of EPCs in Ischemic Stroke Animals

7.6.1 Transplantation Routes for EPCs

The optimal transplantation route for EPCs following ischemic stroke may be important for the therapeutic efficacy. The two routes mostly used for the transplantation of EPCs in stroke are intracerebral and intravascular injections. They each have their own advantages and disadvantages. EPCs intracerebrally injected into the peri-infarct area may be immediately involved in incorporating newly formed vessels or secreting trophic factors to support endogenous repair processes, especially in permanent ischemic stroke to bypass the occlusion of blood vessels. However, invasive injury to the brain raises safety issues.

Intravascular injection either through veins or arteries has minimal invasive injury potential for systematical cell distribution, as well as the far-flung secretion of neuroprotective, pro-angiogenic and immunomodulatory factors (Misra et al. 2012). Intravenously grafted cells can follow a chemokine generated gradient formed by the injured brain and penetrate through the BBB (Guzman et al. 2008),

and grafted cells do not have to be near the lesion to be effective (Borlongan et al. 2004). However, very few cells have been found to integrate into the infarct area. The majority of cells became stuck in the lung, liver, and spleen after intravenous administration. Intra-arterial delivery, in contrast, overpasses the peripheral filtering organs, leading to higher cell engraftment to the brain (Li et al. 2010; Zhang et al. 2012), and greater efficacy (Kamiya et al. 2008; Pendharkar et al. 2010). There is a concern that intra-artery transplanted cells can stick together and cause microemboli, including lethal pulmonary emboli or a reduction in cerebral blood flow, which is associated with microstrokes (Walczak et al. 2008).

In preclinical experimental stroke, intravascular injections are usually used, and they are applied through the tail vein (Zhang et al. 2002; Chen et al. 2012; Nih et al. 2012; Chen et al. 2013d; Decano et al. 2013; Qiu et al. 2013), femoral vein (Moubarik et al. 2011; Pellegrini et al. 2013), jugular vein (Fan et al. 2010; Li et al. 2013), and internal carotid artery (Ohta et al. 2006) (Table 7.4). Despite the different routes used for EPC transplantation, decreased infarct volume, improved neurobehavioral outcomes, increased angiogenesis and neurogenesis, attenuation of endothelial dysfunction, even anti-apoptosis effects have been observed during study. These studies may benefit from both functions of EPCs during cell replacement and enhanced trophic/regenerative support for endogenous repair processes.

However, when considering application in clinical trials, the routes of transplantation of EPCs should be standardized to ease administration. Several clinical studies have been carried out as illustrated in Table 7.5. These studies look into the safety and efficiency of routes for EPC transplantation in human patients, and there is still a lot of work to do in this field.

7.6.2 Modification of EPCs

7.6.2.1 Gene Modification

Considering the paracrine-mediated mechanisms of EPCs, the enhancement of their secretion of trophic factors capacity by the overexpression of related genes would be valuable to magnify the efficacy of EPC therapies in stroke treatment (Chen et al. 2013a). EPCs have been modified by a variety of genes before transplantation and have been reported to enhance functional recovery, these genes include VEGF (Asahara 2007; Gou et al. 2011; Yang et al. 2012), HGF (Song et al. 2009), IGF−1 (Sen et al. 2010), paraoxonase−1 (Wang et al. 2010), CXCR4 (Chen et al. 2012), SDF−1 (Schuh et al. 2012), NO (Chen et al. 2013c), home oxygenase−1 (Long et al. 2013), hypoxia-inducible factor−1α (HIF−1α) (Jiang et al. 2008) and Deltalike−4 (Huang et al. 2013a). There are two major methods for gene transfer systems, viral and nonviral. The most widely used viral vectors for gene transfer are adenovirus and retrovirus. Nonviral methods include the introduction of naked DNA into the target cells and the use of liposomes (Vale et al. 2001).

Table 7.4 Preclinical EPC transplantation studies

Animal models	EPC source	EPC phenotype	Modification	Co-transplantation or not?	Dose	Route and administration time	Outcomes	Mechanisms	Ref
Embolic stroke in mice	BM	NM	No	No	2×10^6	IV via tail vein, 4 weeks before stroke	↑neovascularization	↑angiogenesis and vasculogenesis	(Zhang et al. 2002)
tMCAO in rats	BM	NM	No	No	2.5×10^5	IA, immediately after stroke	↓infarct volume and neurological deficits	↑eNOS, VEGF, IGF-1; ↓endothelial dysfunction	(Ohta et al. 2006)
pMCAO in rabbits	BM	UEA-1, acLDL	No	No	3×10^7	IV, 1 day after ischemia	↓infarct volume and neurological score	↓tunel$^+$ cells; ↑vessel density↑angiogenesis	(Chen et al. 2008)
1 h tMCAO in mice	PB	KDR$^+$/VE-cadherin$^+$/vWF$^+$/Tie−2$^+$; UEA, acLDL	No	No	1×10^6	IV via left jugular vein, 1 h after tMCAO	↓ischemic infarct volume and neurological deficits	↑angiogenesis	(Fan et al. 2010)
tMCAo in rats	PB	CFU-EC	No	No	4×10^6	IV via femoral vein, 1 day after MCAO	↓apoptosis and reactive astrogliosis; ↑capillary density and neurogenesis	↑IGF-1, VEGF secretion	(Moubarik et al. 2011)
pMCAO in type 2 diabetic mice	BM	CD34$^+$/VEGFR-2$^+$/lectin$^+$; acLDL	CXCR4 gene modified	No	2×10^5	IV via tail vein, 2 h after MCAO	↑CBF; ↓infarct volume and neurological score	↑angiogenesis and neurogenesis	(Chen et al. 2012)
Distal MCAO in mice	UCB	CD34$^+$	No	SMPCs, 2.5×10^5	2.5×10^5	IV via tail vein, 1 day after MCAO	↑angiogenesis, vascular remodeling	maintenance of neurogenesis and neuroblast migration	(Nih et al. 2012)

Table 7.4 (continued)

Animal models	EPC source	EPC phenotype	Modification	Co-trans-plantation or not?	Dose	Route and administration time	Outcomes	Mechanisms	Ref
TBI in rats	Spleens	NM	Labeled with SPIO	No	10^6	IV via tail vein, at 6–12 h after TBI	↑CBF	↑microvascular density	(Chen et al. 2013d)
Stroke-prone Rats	PB	$CD45^-$/$CD34^+$/KDR^+	No	No	NM	IA via tail vein, —	Attenuated progression and delayed stroke onset	Maintain vascular healthy	(Decano et al. 2013)
tMCAO in rat	UCB	$CD133^+$	No	No	1×10^7	IV, 1 day after tMCAO	↓infarct volume	↑endogenous proliferation, angiogenesis, and neurogenesis	(Iskander et al. 2013)
tMCAO in rat	UCB	CFU-EC	No	EPO (2500 UI/kg per day × 3)	5×10^6	IV via femoral vein, 1 day after tMCAO		↓apoptosis; ↑angiogenesis and neurogenesis	(Pellegrini et al. 2013)
tMCAO in rat	BM	$CD31^+$/$CD34^+$/$CD133^+$/$Flk\text{-}1^+$	No	No	10^6	IV via tail vein, immediately and 12 h after reperfusion	↓I/R injury	↓caspase-3 activity, Bax and NF-κB, ↑Bcl-2 expression; anti-oxidative and anti-apoptotic properties	(Qiu et al. 2013)

BM bone marrow; *PB* peripheral blood; *UCB* umbilical cord blood; *t/pMCAO* transient/permanent middle cerebral occlusion; *TBI* traumatic brain injury; *CFU-EC* outgrowth colony forming unit-endothelial cell; *SPIO* super-paramagnetic iron oxide; *IV* intravenous, *IA* intra-artery; *acLDL* acetylated low density lipoprotein; *EPO* erythropoietin; *SMPCs* smooth muscle progenitor cells, *CBF* cerebral blood flow; *I/R* ischemia/reperfusion; *NM* not mentioned

Table 7.5 Studies on clinical EPC transplantation

Phase	Patients' symptoms	No. patients	EPC source	EPC markers	Dose	Route	Outcome measures	Status	Location	NTC identifier
I/II	18–80 years, ≤7 days of after stroke; NIHSS≥7 at day 7	NM	BM		2.5×10^6/kg	IV	Number of adverse events; Changes in functional outcomes	Recruiting	China	NCT01468064
I	35–75 years, ≥6 and ≤60 months after stroke	NM	UCB	$CD34^+$	$2–8 \times 10^6$/patient	IC	Change from baseline in NIHSS and brain Image	Not yet recruiting	China	NCT01438593
II	35–70 years, ≥6 and ≤60 months after stroke, NIHSS (9–20)	30	PB	$CD34^+$	$2–8 \times 10^6$/patient	IC	NIHSS, European stroke scale, Barthel index and Mini-Mental State Examination, MRI and CT scans	Completed	China	NCT00950521
I/II	30–80 years, ≤7 days of onset, NIHSS≥8	10	BM	$CD34^+$	NM	IA	Safety, Modified Rankin Score, NIHSS	Recruiting	United Kingdom	NCT00535197
—	≥30 years, ≥6 months and ≤5 years after stroke, NIHSS (9–20)	36	PB	$CD34^+$	Plus G-CSF	IC	Not mentioned	Unknown	China	NCT01239602

BM bone marrow; *PB* peripheral Blood; *UCB* umbilical cord blood; *NIHSS* the national institutes of health stroke scale; *IV* intravenous; *IC* intracerebral; *IA* intra-artery; *MRI* magnetic resonance imaging; *CT* computed tomography;—Not mentioned

EPCs modified by VEGF gene show significantly enhanced neovascularization, even when ten times fewer cells were infused (Asahara 2007), and promote vascular regeneration of ischemic flaps (Yi et al. 2006). In ischemic hindlimb model, transfection of VEGF or heme oxygenase−1genes into EPCs significantly increased the number of differentiated ECs, blood perfusion levels and neovascularization compared to the bare EPCs (Yang et al. 2012; Long et al. 2013). Transfection of EPCs with other genes, such as IGF−1 (Sen et al. 2010), SDF−1 (Schuh et al. 2012), NO (Chen et al. 2013c) and Deltalike−4 (Huang et al. 2013a) genes, to treat ischemic myocardial injury show cell protective and myocardial regeneration effects and functional neovascularization recovery. EPCs modified by paraoxonase−1 genes are potentially valuable in the treatment of atherosclerosis (Wang et al. 2010). Transfection of HGF genes enhances EPC function and improves EPC transplantation efficiency by decreasing neointima formation and increasing reendothelialization for balloon-induced arterial injury (Song et al. 2009).

Some investigators tried to transfect multiple genes into EPCs by using retroviruses to encode both tPA and heparin. Local transplantation of engineered EPCs in a balloon-injured carotid artery model attenuates reendothelialization of angioplasty-injured arteries, but fails to inhibit neointima proliferation (Griese et al. 2003a). In experimental stroke models, only one study found that the transplantation of *CXCR4* gene-modified EPCs reduces cerebral ischemic damage and promotes repair in diabetic mice, and that modified EPCs show better therapeutic effects for ischemic stroke than unmodified EPCs (Chen et al. 2012).

Until now, there have been no clinical trials using gene-modified EPC therapy for the treatment of stroke. It is important to confirm the safety and efficacy of delivering exogenous genes into patients by modifying EPCs. The main concern is the possibility of tumorigenesis after gene delivery. Although exogenous genes are transferred into EPCs rather than to host cells, viral vectors may increase the risk of genotoxicity by insertional mutagenesis and the activation of adjacent oncogenes. To avoid malignant transformation in clinical patients, the vector should be designed for self-inactivation and only contain nonviral, physiologic promoter/enhancer elements (Payen and Leboulch 2012). Second, the therapeutic genes may serve different functions during different pathological stages. For example, SDF−1 plays a key role in promoting angiogenesis and neurogenesis during development (Mithal et al. 2012; Virgintino et al. 2013) and can recruit EPCs towards ischemic lesions for reendovascularization (Fan et al. 2010). Blocking SDF−1/CXCR4 interaction suppresses inflammatory responses and reduces brain infarction in the acute phase of ischemic stroke (Huang et al. 2013b; Ruscher et al. 2013), which indicates that SDF−1 is an inflammation initiator and exaggerates the BBB leakage and ischemic lesions. Whether SDF−1-overexpressing cells could exhibit a similar deterioration effect is unknown, but such studies are fundamental in calling attention to the administration paradigm of EPC gene modified therapy. Third, most completed and ongoing clinical trials employ autologous EPCs for transplantation; the exogenous gene expression in EPCs is time consuming and unavoidably delays cell transplantation. Further studies should be carried out on the effects of delivering

gene-modified EPCs in a later period after stroke or the transplantation ex vivo of expanded EPCs from allogenic sources, which allows for transformation and *in vitro* expansion of EPCs before transplantation (Chen et al. 2013a).

7.6.2.2 Preconditioning EPCs

In addition to the exogenous gene modification of EPCs, investigators have been trying to manipulate endogenous mechanisms for optimizing the therapeutic potential of cell-based stroke therapy by pre-treating EPCs before transplantation. Various factors seem to influence the number of EPCs and their functions, both in experimental stroke models and in clinical patients (Table 7.3). The hypoxia induced by HIF−1α and trophic growth factors such as BDNF, glial cell line-derived neurotrophic factor (GDNF), VEGF and its receptor FIK−1, EPO and its receptor EPOR, SDF−1 and its receptor CXCR4, enhance EPC proliferation, mobilization and the homing to ischemic lesions involved in the repairing process (Kalka et al. 2000b; Vale et al. 2001; Yamaguchi et al. 2003; Bennis et al. 2012). Increased HIF−1α and its downstream genes play central roles in hypoxia-induced defense responses (Ogle et al. 2012). Ischemic preconditioning increases EPC numbers to attenuate partial nephrectomy-induced ischemia/reperfusion injury (Liu et al. 2013a).

VEGF is an important humoral factor for EPC mobilization/differentiation, which is supported by the correlation between the increase in VEGF serum concentration and the increase in circulating EPCs (Sobrino et al. 2012a). EPO stimulates normal EPC-mediated endothelial turnover and improves cardiac microvascularization and function in the presence of ischemia (Westenbrink et al. 2008). Pretreatment of EPCs with EPO before transplantation enhances their angiogenic potential (Bennis et al. 2012). SDF−1 pretreatment during EPC expansion stimulates the adhesion of EPCs to ECs and augments the efficiency of EPC-based cell therapy for ischemic diseases (Zemani et al. 2008). The hormone melatonin stimulates the protective effect of EPCs in acute ischemic kidney injury (Patschan et al. 2012). Exposure to sub-lethal hypoxia can significantly increase the tolerance and regenerative properties of stem/progenitor cells *in vitro* and after transplantation for other cell types (Francis and Wei 2010; Wei et al. 2013; Yu et al. 2013).

Estradiol preserves the integrity of ischemic tissue by augmenting the mobilization and incorporation of EPCs into sites of neovascularization by the eNOS-mediated augmentation of MMP−9 expression in the BM (Iwakura et al. 2006). Angiotensin-converting enzyme 2 (ACE2) improves EPC functions, by regulating eNOS and Nox pathways, enhancing the efficacy of EPC-based therapy for ischemic stroke (Chen et al. 2013b). Other methods, such as the pretreatment of EPCs with extracorporeal shock waves (Lee and Kou 2012) or magnetic bionanoparticles (Li et al. 2013) to enhance the homing and functions of EPCs may also be promising and novel strategies. It is expected that the preconditioning strategy will be further explored due to its potential to enhance the benefits of EPC-based transplantation therapies in stroke therapy (Liu et al. 2013b).

7.7 Pros and Cons in the Application of EPCs in Clinical Trials

EPC transplantation in stroke has pros and cons. As mentioned above, EPCs have shown much potential for stroke therapy either through directly differentiating into mature ECs to replace damaged tissue or by secreting trophic factors to enhance the endogenous repairing processes. Additionally, EPCs can be derived from a variety of sources including PB (Medina et al. 2010), BM (Kwon et al. 2010), cord blood (Li et al. 2013), spleen (Wassmann et al. 2006), adipose tissue (Planat-Benard et al. 2004), and the liver or intestines (Aicher et al. 2007). Ethical limitations are avoided because fetal or embryonic tissues are not necessary sources. A lot of experience in administration of HSCs in clinical treatment of patients with leukemia (Rouhl et al. 2008) shows it is not necessary for autogenous transplanted cells. It allows plenty of time for ex vivo expanded EPCs to be cultured, pretreated or even gene modified, so as to enhance therapeutic capacity when transplanted *in vivo*. However, there are still difficulties that need to be resolved. As EPCs can be cultured by many methods and derived from different sources, and they bear both the characteristic of hematopoietic and endothelial cells, there are no specific markers to identify them and they may also be contaminated by other cell lines like lymphocytes, macrophages or other dendritic cells (Ishikawa and Asahara 2004). Gene expression profiles may also change during EPC culturing (Gremmels et al. 2011). Whether the exogenous gene modified EPCs increase malignant transformation in clinical patients still needs to be further explored.

7.8 Problems Need to be Clarified for the Treatment of Patients

7.8.1 Evaluation of Clinical Safety

Preclinical studies have shown that EPC transplantation is beneficial for functional outcomes without showing side effects, such as enhancing inflammatory responses or forming teratoma. However, to fully ensure the safety of transplanting EPCs in clinical patients, clinical studies have been carried out. A small pilot study suggested that intravenous infusion of autologous EPCs was safe and improved exercise capacity in children with idiopathic pulmonary arterial hypertension (Zhu et al. 2008). EPC transplantation in 20 patients with acute myocardial infarction showed no incensement in the levels of inflammatory markers or troponin T (a marker for cardiac ischemia) (Assmus et al. 2002). Thus, in this small number of patients, EPCs neither seem to stimulate the inflammatory response nor increase ischemia. A variety of clinical studies has also shown that autologous BM stem cell and mesenchymal stem cell transplantation in stroke patients showed nothing

related to abnormal cell growth or tumorigenesis, deteriorated functional outcomes or venous thrombuses (Suarez-Monteagudo et al. 2009; Lee et al. 2010; Honmou et al. 2011; Friedrich et al. 2012; Moniche et al. 2012).

EPCs can secret inflammatory factors such as IL−8 and monocyte chemoattractant protein−1 (MCP−1) (Hur et al. 2004; van der Strate et al. 2007), which might recruit monocytes and macrophages to aggravate ischemia. Currently, several clinical trials (clinicaltrials.gov identifier: NCT00950521; NCT01468064; NCT00535197) are trying to evaluate the safety and efficacy of autologous EPC transplantation in ischemic stroke. EPC transplantation cannot be routinely performed on patients for the treatment of stroke before larger clinical trials further ensure their clinical safety.

7.8.2 Identifying Acceptable Patients for EPC Transplantation

No treatment is appropriate for all stroke patients therapy. Therefore, establishing a criterion for choosing suitable patients for EPC transplantation is vitally important. Stroke patients range in age from 30–80 years old; age should be something to consider because elder patients tend to be suffering from other diseases such as hypertension, diabetes mellitus and dyslipidemic syndromes. Patients with these syndromes show endothelial dysfunction and decreased EPC numbers (Rouhl et al. 2012). It might be difficult for this kind of patient to receive autologous EPCs, which may lose their functional therapeutic effects after transplantation. Methods of modifying EPCs to increase their vasculogenic potential or allogenic EPCs from healthy people may provide options.

Studies have shown that the pathology of stroke in young and aged rats are not identical. For example, after intracerebral hemorrhage, aged rats showed a wider spread of activated microglia/macrophages around the parenchyma and higher astrocyte activity than young rats (Wasserman et al. 2008). Another study showed that aging mice had significantly less edema formation after stoke (Liu et al. 2009). In addition, EPCs are critical components of tumor angiogenesis (Nolan et al. 2007); therefore, EPC transfusion to patients with tumors should be avoided. Considering estrogen has the capacity to promote EPC proliferation (Tan et al. 2012), men and women may respond differently to EPC-based treatments. Whether EPC treatment would have the same efficacy in males and females needs to be considered further.

Infarct location and volume are other factors for determining a patient's suitability for cell transfusion. Preclinical studies in EPC transplantation in stroke (Table 7.4) and different animal models may result in different infarct location and volume in striatum, cortex or both. EPC transplantation shows improvement in functional recovery; however, we cannot exclude the possibility that it may not be as effective in clinical patients. Scoring patients with different lesions, which are usually determined by magnetic resonance imaging (MRI), choosing suitable patients and accordingly giving the appropriate EPC treatment is necessary.

7.8.3 Time, Dose, Route and Type of EPCs for Transplantation

7.8.3.1 Time of EPC Transplantation

Preclinical studies provide various time points to deliver EPCs (Table 7.4). However, the optimal time for transplantation after a stroke is still unclear. After stroke onset, the microenvironment in the brain changes dramatically (Moskowitz et al. 2010). The optimal timing of delivery depends on EPC mechanisms of action, which could replace the damaged cells and promote the endogenous repair process by paracrine effects. If the treatment strategy focuses on cell survival and later cells integrate into the damaged tissue to replace the dead cells, cell survival is extremely important and transplanting during the recovery phase of stroke to avoid inflammation could be beneficial. Otherwise, if the treatment acts to enhance the endogenous repair process or protective mechanisms by paracrine effects, acute phase delivery is critical (Hayashi et al. 2003; Carmichael 2006). Preclinical studies of the delivery of EPCs were done either immediately after stroke or from 1 h to 1 day after stroke, which showed functional recovery in animals. However, a systematic analysis of transplantation timing and its effect on functional recovery has not been done.

7.8.3.2 Dose Injection of EPCs

In addition, as we move towards clinical trials, cell dosage becomes an important question to consider. Different cell dosages have been applied during preclinical trials (Table 7.4). Cell dosages influence cell viability after transplantation; fewer cells may not be enough to function as a therapeutic treatment, while an excessive amount of cells may result in side effects such as inflammation, teratoma or microembolus. Ongoing clinical studies are designed to use $2–8 \times 10^6$ EPCs to treat stroke patients (clinical trials.gov identifier: NCT01468064; NCT01438593; NCT00950521), and the safety and efficacy of EPC therapies are not yet clear.

7.8.3.3 Routes of Administration

Studies have reported functional recovery using the intravenous and intracerebral delivery of EPCs. All routes resulted in cells targeting the lesion, but more cells were found at the lesion with intracerebral delivery than with intravenous delivery (Jin et al. 2005). Preclinical studies choosing intravenous or intra-artery delivery of EPCs have shown functional recovery. Clinical studies plan to apply either intravenous or intracerebral delivery routes. In regards to EPC transplantation in clinical trials, intravenous infusion should be the optimal route because intracerebral injection is invasive and inconvenient, and intra-artery delivery may cause embolisms (Borlongan et al. 2004).

7.8.3.4 Types of Used EPCs

Different types of EPCs play specific roles, with early EPCs protecting damaged tissue by secreting amounts of pro-angiogenic factors, and late EPCs integrate in to host vessels to replace damaged ECs. However, it is still difficult to define EPCs, because they have multiple markers. Investigators are trying to identify, isolate and expand EPCs using their normal markers, such as CD34, KDR, and CD133, but related cell types might bear the same markers. All the ongoing clinical trials use CD34 to identify EPCs, which might not be sufficient. Optimizing the isolation and identification of EPCs from patients is still a critical problem.

7.8.4 Bio-Distribution and Persistence of EPCs

When EPCs are transplanted into ischemic animals or patients, it is crucial to monitor where the EPCs travel and into what cell types they differentiate. This helps us to understand how these cells mediate functional recovery. Therefore, dynamic non-invasive tracking of grafted EPCs *in vivo* is necessary. Optical imaging, MRI and nuclear imaging are potential imaging strategies and MRI is most often used for the dynamic tracking of grafted EPCs *in vivo*. For the tracking of exogenous EPCs *in vivo*, the grafted cells must be labeled with contrast agents *in vitro* before transplantation so that they are distinguishable from the host tissue. Molecular probes such as transferrin have successfully been used to tag putative stem cells followed by high-resolution MRI to track the homing of cells (Weissleder et al. 2000). Currently, gadolinium rhodamine dextran (GRID) and superparamagnetic iron oxide (SPIO) are two groups of commonly used contrast agents. Some studies have been successful in long-term monitoring EPCs using MRI in a rat hindlimb ischemic model (Agudelo et al. 2011; Agudelo et al. 2012). However, little work has been done on the dynamic tracking of EPCs in ischemic stroke and it calls for much attention in order to provide fundamental data for its application in clinical trials.

Conclusions

Preclinical studies have shown great promise for EPC transplantation as a therapy for stroke. Beneficial effects from EPC transplantation have been observed, including functional improvement, increased neovascularization and decreased apoptotic cells. However, there are many fundamental unsolved problems, mentioned above, and relevant clinical trials are needed. A guideline called Stem Cell Therapy as an Emerging Paradigm for Stroke (STEPS) in 2009 (The STEPS Participants, 2009) has been drawn up. To some extent, this guideline can help scientists in all fields collaborate to accelerate the use of EPC transplantation in clinical patients

for those who might benefit from EPC therapy. Currently, EPC transplantation for stroke treatment in clinic is only a vision, preclinical studies and clinical research are needed to maximize the therapeutic benefit and minimize the risks of EPCs transplantation in stroke.

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