Review

Endothelial Progenitor Cells and Kidney Diseases

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Key Words

Endothelial progenitor cells • Cardiovascular disease • Acute kidney injury • Chronic kidney disease • Glomerulonephritis • Sepsis • Renal transplantation • Microvesicles • Atherosclerosis

Abstract

Endothelial progenitor cells (EPC) are bone marrow derived or tissue-resident cells that play major roles in the maintenance of vascular integrity and repair of endothelial damage. Although EPCs may be capable of directly engrafting and regenerating the endothelium, the most important effects of EPCs seem to be depended on paracrine effects. In recent studies, specific microvesicles and mRNAs have been found to mediate the pro-angiogenic and regenerative effects of EPCs on endothelium. EPC counts have important prognostic implications in cardiovascular diseases (CVD). Uremia and inflammation are associated with lower EPC counts which probably contribute to increased CVD risks in patients with chronic kidney disease. Beneficial effects of the EPC therapies have been shown in studies performed on different models of CVD and kidney diseases such as acute and chronic kidney diseases and glomerulonephritis. However, lack of a clear definition and specific marker of EPCs is the most important problem causing difficulties in interpretation of the results of the studies investigating EPCs.

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Introduction

Endothelial progenitor cells (EPCs) are stem cells that play major role in the maintenance of vascular integrity and repairment of endothelial damage [1, 2]. EPC deficiency and dysfunction may contribute to increased risk of cardiovascular disease (CVD) observed in patients with chronic kidney disease (CKD) [3-6]. Furthermore, EPCs may also be important in the pathogenesis of various kidney disorders including ischemic acute kidney injury (AKI), sepsis, CKD, glomerulonephritis, acute and chronic rejection in patients with renal

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transplantation (RT). Herein we aimed to summarize the possible effects of EPCs in CVD and kidney diseases. Investigative therapeutic modalities using EPCs were also discussed.

Basic Concepts about EPCs

Vasculogenesis in adult life: emergence of EPCs

Until recently, new vessel formation in adult life have been considered to be through “angiogenesis” by sprouting of endothelial cells from pre-existing blood vessels. However, during embryonic development, stem cell-mediated new vessel formation is called “vasculogenesis”. In the light of new evidences, vasculogenesis has been shown to occur also in adult life. The first evidence of postnatal neovascularization was demonstrated by Asahara et al. [7]. In this study, CD34+ and fetal liver kinase-1+ (Flk1+) (vascular endothelial growth factor receptor: VEGFR) progenitor cells isolated from the peripheral blood were shown to incorporate into the sites of ischemia in an immunodeficient mice model with hindlimb ischemia. The term EPC was first used in this study and EPCs were suggested to be useful for collateral vessel growth in ischemic tissues.

Definition of EPC

EPCs are defined as non-endothelial cells that are capable of differentiating into endothelial cells. EPCs have characteristically two features: clonal expansion and stemness (ability to proliferate and resistance to stress) [8]. However, in experimental point of view, EPCs are frequently defined as mononuclear cells that are: 1) able to adhere to matrix molecules such as fibronectin and 2) double positive for acetylated low-density lipoprotein (acLDL) and Ulex europaeus agglutinin (UEA-1) lectin in cell culture studies [7].

Cell surface markers are also used to characterize EPCs in an increasing frequency however a single specific marker or combinations of markers for EPCs have not yet been identified. Most commonly used markers of EPCs include CD34, VEGFR-2 and CD133. These markers are usually used in double (CD34+/VEGFR2+, CD133+/VEGFR2+) or triple (CD34+/CD133+/ VEGFR2+) combinations. Lack of a specific marker, scarcity of EPC in the circulation and the presence of several common characteristics between EPCs and other hematopoietic and mature endothelial cells cause difficulties in interpretation of the results of the studies investigating EPC [9].

Types of EPCs: early and late EPCs

Two different populations of EPCs named as early and late EPCs have been suggested according to time needed for cultivation. “Early EPCs” (or colony-forming unit-endothelial cells: CFU-ECs) grow in cell culture in 4-7 days however “late EPCs” (or endothelial colony-forming cells: ECFCs) appear in 14 to 21 days in culture [10].

Early EPCs have limited capacity to proliferate and these cells indirectly induce angiogenesis in 3 ways: 1) by arranging the perivascular microenvironment by secreting paracrine factors [11, 12]; 2) by secreting vasomodulatory microvesicles containing micro-RNAs (miR-126 and -296) [13]; 3) by communicating with mature endothelial cells by microtubular organelles (called nanotubes) [14]. Thus this type of EPC is sometimes named as “hematopoietic cells with proangiogenic activity”. In contrast, late EPCs have high capacity of proliferation and they directly engraft into the injured site and regenerate into mature endothelium [11, 15]. Although early and late populations of EPCs have different ways of function, both of these cell types lead to enhanced neovascularization during hindlimb ischemia [11, 16, 17]. In fact, early and late EPCs may act together and support each other in normal vascular physiology [18, 19].

In the study by Patschan et al., effects of early and late EPCs were compared in an ischemic AKI model [20]. Late EPCs were capable of reducing interstitial fibrosis in the mid- to long-term however peritubular capillary loss could not be prevented. In this study, late EPCs were

not as efficient as early EPCs in preventing mice from ischemia-induced AKI. Although some subsets of EPCs may be capable of directly engrafting and regenerating endothelium, the most important effects of EPCs seem to be depended on paracrine effects.

Paracrine effects of EPCs: extracellular vesicles

Extracellular vesicles (EV) are small membrane particles that play important roles in cellular communication through transportation of various molecules such as proteins and micro-RNAs [21]. EVs can be divided into 2 groups: microvesicles and exosomes. Microvesicles are of 100- to 1000-nm diameter and they are formed from the plasma membrane through direct shedding. Exosomes are smaller than microvesicles with a diameter of 40- to 100-nm which are released by exocytosis [22]. EVs secreted from EPCs have previously been reported to activate angiogenesis by transfer of mRNAs to mature endothelial cells leading to prevention from ischemic AKI [13, 23]. In a rat model of ischemia-reperfusion injury, possible effects of EPC-derived-microvesicles on prevention of AKI have been investigated [13]. Microvesicles were found to contain microRNAs (namely microRNA-126 and microRNA-296) that modulate proliferation, angiogenesis and apoptosis. After intravenous injection, these microvesicles were found to localize to peritubular capillaries and tubular cells and enhance tubular cell proliferation and decrease leukocyte infiltration leading to protection from AKI. Furthermore, these microvesicles halted the progression of CKD by inhibiting glomerulosclerosis and tubulointerstitial fibrosis. In this study, total microRNA depletion by Dicer knockdown and RNase digestion diminished the protective effects of EPC-derived microvesicles. In another study by Cantaluppi et al. [24], EVs were injected intravenously to rats with anti-Thy1.1 glomerulonephritis and these EVs were shown to have protective effects by amelioration of mesangial injury caused by antibody and complement-mediated mechanisms.

Late EPCs may have also paracrine effects by the way of exosomes [25, 26]. In the study by Vinas et al. [25], infusion of exosomes (enriched in miR-486-5p) derived from ECFC led to functional and histologic protection in a mice model of ischemic AKI. In a study by Burger et al. [26], effects of ECFCs and their EV were investigated in an ischemic AKI model. EPC-derived exosome injection was found to decrease renal injury. In cultured endothelial cells, ECFC-derived exosomes ameliorated endothelial cell apoptosis through caspase-3 inhibition.

Where do EPCs come from?

There are conflicting data about the possible sources of EPCs, in several studies EPCs were found to have bone marrow (BM) origin, in other studies EPCs were shown to be tissue resident cells. In a mouse model of selective endothelial injury by Hohenstein et al. [27, 28], BM-derived cells carrying markers of EPCs were found in injured kidneys and these cells were shown to contribute to subsequent healing of endothelial lesions. However, in another study, Sradnick et al. investigated the potential role of EPCs in renal endothelial cell repair [29]. In this study, extrarenal cells were not found to replace any mature endothelial cells suggesting that endothelial repair depends on local mechanisms in acute endothelial injury model. In another words, instead of giving rise to mature EPCs inside the kidney, cell therapy with EPCs were suggested to indirectly take part in the endothelial repair process via angiogenic cytokines. Authors suggested naming these cells als “pro-angiogenic cells” instead of EPCs.

Methods used in EPC studies

EPCs can be studied by 3 strategies; 1- direct cell counting with flow cytometry, 2- cultivating EPCs in fibronectin-coated plates and counting the cells growing in the culture, 3- combination of these 2 strategies, cell sorting of EPCs and then cultivation of these cells (Fig.1).

Flow cytometry is advantageous because direct counting of the cells without complicated culture steps makes this approach reliable. However there is no consensus on the exact definition of EPC in terms of surface markers and it is not possible to perform functional tests in flow cytometry. These are the main limitations of the flow cytometry.

The main advantage of the culture method is that it makes the functional tests possible. Migratory activity, tube formation capability as an *in vitro* model for angiogenesis and adhesion capacity to endothelial cells and to extracellular matrix molecules are possible functional tests. Indeed, even if specific functional studies are not performed, culture method suggests important functional characteristics of EPCs

such as capabilities of differentiation and proliferation. However cell culture consists of complicated *in vitro* steps making this method vulnerable to flaws. Since, very high concentrations of growth factors are needed in culture method when compared to *in vivo*, cultivated EPCs may not represent the EPCs in normal physiology.

Combination of these two strategies is also possible [30-32]. EPCs may be selected by fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS) and then these cells may be cultivated. In this method, both the EPC counts and functional test may be performed.

Direct cell counting with flow cytometry is generally accepted as the method of choice for determination of EPC number [33]. However, in certain situations, EPC dysfunction rather than absolute EPC count may be more important [34]. In such cases, investigation of functional and clonogenic properties of EPCs may be required necessitating cell culture methods. Performing both the flow cytometry and cell culture methods may provide further complementary data.

Fig. 1. Culture and flow cytometry methods for counting and cultivation of EPCs (FACS: Fluorescence-activated cell sorting, MACS: Magnetic-activated cell sorting, acLDL: acetylated low-density lipoprotein)

Factors affecting EPC counts

Factors affecting EPC counts are presented in Table 1. In summary, EPC counts and functions are negatively affected by uremia, inflammation and other risk factors for atherosclerosis such as smoking, diabetes mellitus and hypercholesterolemia [35, 36]. Acute tissue injuries, certain drugs such as statins and growth factors and erythropoietin are associated with increased EPC counts and/or functions [37-39].

EPC studies in various disease models

EPCs and cardiovascular diseases: prognostic importance of EPCs

EPC counts may be reliable markers of vascular health [33, 40]. Decreased EPC counts may hinder re-endothelization after vascular injury leading to endothelial dysfunction, intimal hyperplasia and eventually thrombosis [41-43]. Vasculoprotective role and prognostic importance of EPCs in atherosclerotic diseases have been shown in “Endothelial Progenitor Cells in Coronary Artery Disease” study [33]. In this study, effects of CD34+/kinase insert domain receptor (KDR)+ cell counts on cardiovascular end-points were investigated in patients with coronary artery disease (CAD). After adjusting for vascular risk factors, drug treatments and comorbidities; high EPC counts were significantly associated with lower risk of cardiovascular mortality, the need of revascularization and hospitalization. Indeed, circulating EPC counts have been shown to predict the impairment of vascular reactivity far better than Framingham risk factor scoring [40]. Other studies have also shown negative correlations between EPC counts and cardiovascular risk factors and mortality [44, 45]. CD34+/KDR+ cells were found to be reduced by 50% in patients with CAD compared to healthy controls. Furthermore EPCs isolated from patients with CAD were found to have impaired migratory response [44]. In a study performed on subjects with cardiovascular

Table 1. Factors affecting EPC counts and functions. (ACE: angiotensin converting enzyme, ARB: angiotensin-2 receptor blockers, VEGF: vascular endothelial growth factor, GM-CSF: granulocyte-macrophage colony stimulating factor, SDF-1: stromal cell-derived factor-1, HGF: hepatocyte growth factor, eNOS: endothelial nitric oxide synthase, ADMA: asymmetric dimethyl-arginine)

Increased EPC counts/functions	Decreased EPC counts/functions
Hypoxemia [124]	
Exercise [125]	
Heart failure (early phase) [126]	Advanced age [142]
Acute myocardial infarction [127]	Chronic kidney disease [46,49,50,56,57]
Stroke [128]	Diabetes mellitus [36]
Local tissue ischemia [129]	Hypercholesterolemia [60]
Vascular injury [130]	Heart failure (late phase) [127]
Ischemic preconditioning [131]	Hyperhomocysteinemia [143]
	Increased inflammation (high CRP levels) [59]
<u>Drugs:</u>	Inflammatory diseases (rheumatoid arthritis) [144]
Statins [1,132], erythropoietin [38,39], ACE inhibitors [133], ARBs [134], rosiglitazone [135] Sitagliptin [136]	Smoking [145]
<u>Cytokines and growth factors:</u>	<u>Drugs:</u>
VEGF [137], GM-CSF [130], anjioipoietin-1 [138], SDF-1 [139], HGF [140], melatonin [141]	Rapamycin [146]
<u>Genetic modifications:</u>	<u>Cytokines and growth factors:</u>
Overexpression of eNOS [115]	Indoxyl sulphate [147]
Overexpression of heme-oxygenase 1 [115]	P-cresol [148]
Overexpression of telomerase reverse-transcriptase [116]	ADMA [149]
Deletion of p66ShcA [117]	<u>Genetic modifications:</u>
Downregulation of the Ets1 transcription factor [118]	Sirtuin-1 knockout [150]

risk factors but without a history of CVD, EPC counts were significantly associated with flow-mediated dilatation (FMD) of brachial-artery, an indicator of endothelial function [40]. However in our previous study, EPC counts were not associated with FMD or carotid intima-media thickness in HD patients [46]. We suggested that other uremia-related factors might have overridden the clinical picture concealing the possible effects of decreased EPC counts on endothelial function and atherosclerosis. Decreased EPC counts have also been found to be associated with early and frequent restenosis of dialysis vascular accesses after percutaneous angioplasty [47]. In a 3-year prospective study by Hsiesh et al. [48], decreased circulating EPC counts (defined as CD34+KDR+ cells) were significantly associated with systemic vascular thrombosis and vascular access thrombosis.

EPCs and chronic kidney disease

EPC counts have been reported to be lower in CKD patients [49, 50]. Decreased EPC counts in CKD may be caused by impaired EPC mobilization from BM, depletion of EPCs due to increased demand or sequestration of EPCs in kidneys [51]. In our previous study, we also found that EPC counts determined by cell culture method were significantly lower in HD patients compared to healthy controls [46].

In vitro studies showed that uremic serum caused EPC dysfunction and also impaired differentiation of mononuclear cells to EPC [52]. Indoxyl-sulfate (IS) which is a protein-bound uremic toxin, was found to have a toxic effect on vascular endothelial cells [53] and renal tubular cells [54, 55]. In the study by Lin et al. [56], IS inhibited colony formation and functions of EPCs in a dose dependent manner in patients with CKD.

EPC counts may be inversely correlated with the degree of renal dysfunction. In the study by Surdacki et al. [57], glomerular filtration rate was inversely associated with CD34+/KDR+ cell counts in patients with CAD.

Inflammation is the one of the cornerstones in the pathophysiology of CKD. Uremia-induced inflammation contributes to EPC dysfunction. Proinflammatory cytokines are known to impair EPC functions and differentiation [58, 59]. In our previous study, we found that EPC counts were negatively associated with inflammation [46]. Specifically, EPC number determined by cell culture method was inversely associated with serum TNF- α levels in HD patients. Furthermore, CD34+/VEGFR-2+ cell counts were negatively related to serum interleukin-6 levels.

Other important factors associated with EPC dysfunction in CKD may be the oxidative stress and inadequate nitric oxide (NO) production [60-63]. Increased oxidative stress may lead to impaired differentiation of EPCs to mature endothelial cells and disordered regeneration of injured vascular endothelium [60, 61]. NO synthase which is necessary for EPC mobilization, is inhibited by uremic guanidin compounds resulting in inadequate NO production that leads to impaired mobilization of EPC from BM [64].

While CKD is affecting EPC counts and functions, EPCs may also have influence on the progression of CKD. Possible effect of EPC treatment on the progression of CKD was investigated in a 5/6 nephrectomy model [65]. In this study, adoptively transferred EPCs homed to the injured kidney, decreased inflammation and proteinuria and consequently renal structures and functions were preserved.

Influence of dialysis dose and modalities on EPCs

Dialysis dose is an index of clearance of uremic toxins and it is significantly associated with the prognosis of patients with CKD [66, 67]. HD dose was also found to be associated with EPC functions; higher HD dose was related to recovery of angiogenic functions of EPCs [49]. However thrice weekly HD may not be adequate for the recovery of EPC health. Increasing dialysis dose may be associated with better outcomes. EPC counts and migratory functions of patients under nocturnal HD treatment were comparable with those of healthy controls [68]. Similarly, EPC counts were reported to be normalized in patients treated with high-efficiency peritoneal dialysis modalities [69]. In the study by Yuen et al. [70], early EPCs

were cultured from healthy controls, conventional and nocturnal HD patients and these EPCs were administered into the ischemic hindlimb of rats 1 day after left common iliac artery ligation. EPCs obtained from conventional HD had no effect on ischemia however EPCs from nocturnal HD and healthy controls significantly improved ischemic hindlimb perfusion and capillary density.

Besides the dose of the dialysis, different dialysis modalities may have influence on EPC counts or functions. High-flux HD with online hemodiafiltration (HDF) is well-known to be associated with better middle molecule removal and reduced inflammation [71]. In a cross-over trial by Krieter et al. [72], 18 patients were subjected to 4 weeks of low-flux HD, high-flux HD and HDF. However, in this study, dialysis modalities had no effect on EPC numbers.

EPCs in ischemic AKI models

In an animal model of renal ischemia/reperfusion (I/R) injury, Pang et al. investigated renal artery-derived progenitor cells (RAPC) (defined as CD34+/CD105-cells) obtained from human renal arterial adventitia of radical nephrectomy specimens [73]. RAPC were found to have EPC-like characteristics. Injected RAPC integrated into endothelium after acute ischemia/reperfusion (I/R) injury and decreased serum creatinine levels and albuminuria and improved blood flow. Endothelial migration was improved when injured endothelial cells were exposed to RAPC-derived exosomes containing high levels of miRNA-218.

EPCs may also affect the recovery process of AKI and may reduce the progression to CKD after AKI. In an ischemic AKI model by Pastchan et al. [74], systemic injection of early EPCs decreased serum creatinine and ameliorated interstitial fibrosis. Exposure of endothelial cells to early EPC supernatant led to decreased smooth muscle actin and tubulin expression. Furthermore percentages of cilium-positive cells were increased resulting in reduced endothelial-to-mesenchymal transformation. In this study, authors concluded that endothelial cilia might be suggested as an anti-fibrotic organelle and early EPCs might stabilize cilia integrity and functions in ischemic AKI.

EPCs in sepsis

Sepsis seems to increase the number of circulating EPCs but impair their functions [75-77]. In the study by Becchi et al. [75], EPC numbers were increased in sepsis and furthermore, EPC counts were associated with the severity of the sepsis. Similarly Rafat et al. [76] found that increased EPC counts were associated with the survival of patients with sepsis. In the study by Patschan et al. [77], EPC counts (defined as CD133+/Flk-1+) were also found to be increased in sepsis however patients with increased creatinine levels showed even higher EPC counts. EPC proliferation was investigated by a colony-forming units assay and sepsis was found to significantly impair the proliferative capacity of EPCs. In this study, serum levels of stromal cell-derived factor-1, angiopoietin-2, and VEGF were significantly increased in patients with sepsis. These molecules are known to enhance the mobilization of EPCs suggesting an explanation for increased EPC counts in sepsis [78].

EPCs in glomerulonephritis models

EPCs were also studied in glomerulonephritis models and administration of EPCs was found to have favorable effects on disease processes. In the study by Uchimura et al. [79], possible effects of bone-marrow derived mononuclear cells on glomerular endothelial cell injury were investigated in an anti-Thy-1.1 nephritis model. These cells were enriched in a BM culture after cultivation under conditions that promote EPC and administered only to the left kidney via renal artery. These cells integrated into the glomerular endothelium and took role in the repair processes. Compared to contralateral kidney, endothelial injury was lower in the cell-transferred kidney. Similarly, other several studies with glomerulonephritis models, have shown that BM derived cells were recruited into the kidney and repaired the endothelium leading to decreased endothelial injury [80, 81]. In a study performed on a model of IgA nephropathy [82], EPC administration to rats resulted in decreased proteinuria

and serum creatinine levels. Furthermore, EPC transplantation diminished glomerular extracellular matrix and IgA deposition in the glomeruli slowing the progression of IgA nephropathy.

EPCs in renal artery stenosis models

EPC treatments have been shown to be beneficial in renal artery stenosis (RAS) models. In the study by Chade et al. [83], EPC injection into the stenotic kidney improved microvascular density, renal functions and diminished fibrosis in an experimental RAS model. In another study by Ebrahimi et al. [84], EPC administration resulted in EPC engraftment into the tubular structures leading to improved tubular functions and decreased fibrosis in a swine RAS model.

EPCs in renal transplantation

RT has been shown to improve vascular functions and decrease cardiovascular mortality compared to chronic HD [85-87]. However, there are controversial results about the role and functions of EPCs in RT. In the study by Soler et al. [88], EPC counts and proliferation capacities were found to be decreased in RT recipients compared to healthy controls. Herbrig et al. [89] showed that EPC counts were decreased however EPC functions were improved after RT. Decreased EPC counts in this study could be explained by two reasons: 1- erythropoiesis stimulating agents which are powerful EPC stimulators are usually withdrawn in post-transplant period, 2- immunosuppressive treatments especially cyclosporine and corticosteroids might have impaired the differentiation of EPCs from mononuclear cells. In the study by De Groot et al. [90], EPC counts in RT recipients were similar to healthy controls but significantly higher than uremic patients. In this study, EPC numbers were correlated significantly with the graft function in RT recipients. In the study by Di Marco et al. [91], RT patients were compared to healthy controls in terms of EPC numbers (defined as CD133+/VEGFR2+ and CD34+/VEGFR2+). EPC counts were higher in RT recipients compared to controls. Furthermore in a 5/6 nephrectomy model, possible impact of immunosuppressive drugs on EPC counts was investigated. EPC counts were lower in rats with CKD compared to control animals however administration of immunosuppressives restored the EPC counts in these rats. Authors suggested that these drugs might contribute to increased EPC counts observed in RT patients.

EPCs may also play important roles in the acute and chronic rejection processes in RT patients. In several studies, EPCs were shown to accumulate in renal allograft in cases of rejection as a response to increased expression of adhesion molecules, chemokines and growth factors such as VEGF [92, 93]. Consequently an increased angiogenesis is observed in such situations. In acute diseases such as ischemic nephropathy and in chronic states such as tubulointerstitial fibrosis and aging, microvascular rarefaction is the main pathology in which increased angiogenesis by the way of EPCs may be beneficial [94-97]. However in cases of chronic allograft vasculopathy (CAV), persistent injury leads to accumulation of leukocytes and EPCs that result in a reciprocal process of inflammation and angiogenesis. In such cases, angiogenesis itself increases inflammation and vice versa [98-100]. Thus inhibition of angiogenesis may attenuate the development of CAV [101]. Although chimerism created by the homing of EPCs to allograft's endothelium may be beneficial in acute insults, it may increase indirect pathway of allorecognition leading to immunologic injury in chronic rejection [102, 103]. In the study by Yang et al. [104] performed on an abdominal aortic transplant model, injected EPCs (defined as stem cell antigen-1+/Flk-1+ cells) did not promote re-endothelialization after transplantation, but rather increased endothelial cell damage, leading to transplant arteriosclerosis in the allograft transplantation group. Furthermore, vandetanib- a tyrosine kinase inhibitor acting as a VEGFR inhibitor- was found to reduce transplant arteriosclerosis by inhibiting proliferation, migration and adhesion of EPCs.

Possible adverse effects of EPCs: “Bad” EPCs?

Although EPCs are known to have favorable effects on vascular health, a subset of EPCs may be associated with atherosclerotic plaque instability by the way of increased neovascularization leading to the progression of atherosclerosis [105-109]. In the study by Flammer et al. [110], circulating osteocalcin+ early EPCs were strongly associated with unstable coronary artery disease. Authors suggested that this particular EPC subset might cause abnormal vascular repair.

CD133+ cells belong to a subset of undifferentiated stem cells with controversial role in the pathogenesis of CVD [111]. There are several studies about the role of CD133+ cells in prevention of various CVD with favorable results [112, 113]. However, in other studies, these cells had detrimental effects on cardiovascular health [105-109, 114]. For example intracoronary injection of CD133+ cells was found to increase in-stent restenosis [108]. In another study, CD133+ cell injection increased inflammation and ischemic AKI in a mice model [105]. Neutrophil infiltration and myeloperoxidase activity increased in kidneys and TNF- α levels increased in plasma. In this study, increased TNF- α was suggested to be responsible for the pro-inflammatory effects of CD133+ cells [105]. In parallel to this study, in our previous study performed on HD patients, we found that CD133+ cell counts were associated with increased inflammation determined by serum TNF- α and resistin concentrations [114]. Importantly, in our study, CD133+ cell counts were significantly negatively related to FMD suggesting possible adverse effects of these cell types on endothelial functions.

EPC engineering: Improving EPC functions and delivery

Certain cellular modifications may enhance EPC functions. For example, overexpression of eNOS or heme-oxygenase-1 resulted in improvement of EPC functions [115]. Overexpression of telomerase reverse-transcriptase in EPCs improved neovascularization of ischemic limbs [116]. p66ShcA deletion was shown to reduce glucose-induced oxidative stress and improve the viability of EPCs [117]. In another study, downregulation of the Ets-1 transcription factor increased EPC numbers and differentiation to endothelial cells in a mouse model of metabolic syndrome and type-2 diabetes mellitus [118].

Delivery of EPCs to the desired site of injury is an important issue in therapeutic use of EPCs. There may be some problems in the administration of EPCs: 1) only a small percentage of EPC may be able to home to the kidney [119, 120]; 2) inadequate cellular integrin activation may lead to apoptosis of EPCs if the cells are administered in an aqueous solution [121]. To prevent these possible problems, Ratliff et al. [122] administered EPCs within hyaluronic acid-based hydrogels into the ears or injected EPCs subcapsularly into the kidneys. Post-ischemic kidney functions were significantly improved in both types of administration. This approach of delivery of EPCs within hydrogels was found to be superior to intravenous EPC injection.

Decellularized organ scaffolds have been increasingly used in regenerative medicine. In the study by Wang et al. [123], partial nephrectomy was performed and these kidneys were repaired with decellularized renal scaffolds in rats. Subsequently, EPCs were injected into the rats. With this approach, angiogenesis and microvascular density were found to be higher compared to control group. Decellularized renal scaffolds may provide a convenient microenvironment for EPCs in the repair process after kidney injury.

Future of EPC studies

Since the most important problem in EPC research is the lack of a common EPC definition, further studies should focus on the definition of EPCs. EPCs have been clearly shown to be important predictors in CVD but not in kidney diseases. Possible prognostic aspects of EPC counts and functions should be investigated especially in AKI and RT. Therapeutic roles of EPCs have been studied in various kidney diseases with mostly cell culture and animal models. Further human trials should be performed possibly by engineered EPCs with greater therapeutic benefits. However, possible adverse effects of certain subsets of EPCs such as endothelial dysfunction and increased cardiovascular risks should be kept in mind in human studies.

Conclusion

EPCs are shown to have important roles in the maintenance of vascular integrity and repairment of endothelial damage. Although EPCs may be capable of engrafting and regenerating endothelium, the most important effects of EPCs seem to be depended on paracrine effects. EPC counts have strong prognostic implications in CVD. Importantly, EPCs, as therapeutic agents, have been found to be beneficial for especially AKI. Further human studies investigating EPC therapies for kidney diseases are needed.

Disclosure Statement

The authors declare they have no conflicts of interests regarding the publication of this article.

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