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REVIEW

Dysfunction of Endothelial Progenitor Cells under Diabetic Conditions and its Underlying Mechanisms

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Cardiovascular complications have been major concerns in the treatment of diabetes, and up to 80% of all deaths in diabetic patients are linked to cardiovascular problems. Impaired angiogenesis is one of the most serious symptoms associated with diabetes, resulting in delayed wound healing and lower limb amputation. Endothelial progenitor cells (EPCs), a subpopulation of adult stem cells, are recruited from bone marrow to the injured vessel to promote endothelial regeneration and neovascularization, playing an important role in angiogenesis. Interestingly, several clinical studies have showed that the number of recruited EPCs is reduced and their function is decreased under diabetic conditions, implying that diabetic EPC dysfunction may contribute to defective angiogenesis and resultant cardiovascular complications in diabetes. To recover the functional abilities of diabetic EPCs and to address possible application of EPC cell therapy to diabetic patients, some studies provided explanations for diabetic EPC dysfunction including increased oxidative stress, involvement of the inflammatory response, alteration in the nitric oxide pathway and reduced signals for EPC recruitment. This review discusses clinical evidence of impairment of EPC functions under diabetic conditions and the suggested mechanisms for diabetic EPC dysfunction.

Key words: Angiogenesis, Diabetes-associated cardiovascular complications, Endothelial progenitor cells, EPC Dysfunction

INTRODUCTION

Accelerated cardiovascular complications are one of the most principal causes for disability and death in patients with diabetes mellitus (Creager et al., 2003; Falanga, 2005). The balance between cardiovascular injury and recovery under diabetic conditions, in addition to impaired angiogenesis and endothelial damage are suggested as critical steps in diabetes-associated cardiovascular complications including atherosclerosis, delayed wound healing, thrombosis and hypertension (Beckman et al., 2002; Brem and Tomic-Canic, 2007; Shantsila et al., 2007; Leeper et al., 2010). Endothelial progenitor cells (EPCs) are known to be recruited to the injured site to promote endothelial regeneration, and the role of EPC in the diabetes-associated cardiovascular imbalance has been raised. Interestingly, the number of recruited EPCs as well as their function is decreased in diabetic patients, implying that impaired EPC function may contribute to cardiovascular problems in diabetes (Tepper et al., 2002; Loomans et al., 2004; Gallagher et al., 2007; Shantsila et al., 2007). Supporting these clinical data, several studies have given some explanations for diabetic EPC dysfunction including increased oxidative stress, NADPH oxidase activation, an altered nitric oxide pathway and increases in inflammatory cytokines. It is unlikely that the dysfunction of diabetic EPCs could be explained by a separate independent mechanism, but the complicated patho-physiological changes under diabetes could exacerbate the functional damage of EPCs contributing to retarded reendothelialization and vascular recovery. Nevertheless, the precise understanding of the mechanisms underlying diabetic EPC dysfunction is required to overcome

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diabetes-associated EPC dysfunction and resultant vascular damage, as well as to address the therapeutic potential of EPCs for the prevention and treatment for cardiovascular complications in diabetes. The goal of this review is to provide an overview of the clinical role for EPCs in diabetes-associated cardiovascular complications and the relevant mechanisms for impairment of EPC function under diabetic conditions. Here we discuss the general characteristics of EPCs, the role of EPCs in cardiovascular diseases, EPC dysfunction under diabetic conditions and suggested mechanisms for diabetic EPC dysfunction.

EPCs AND VASCULAR REGENERATION

Since Asahara et al. discovered the vasculogenic properties of the EPC population in 1997, there have been many attempts to expand the therapeutic application of EPCs for vascular regeneration and restoration of vascular function. The exact characteristics and the function of EPCs are still under intensive investigation. Rather than a specific cell type with fixed characteristics, it should be more correct to define EPCs as a heterogeneous cell population with distinct phenotypes, which can differentiate into functionally competent endothelial cells. There have been various opinions regarding whether to include EPCs as stem cells or not. Leeper et al. (2010) categorized EPCs as one of the 'lineage-committed adult stem cells' and Urbich and Dimmeler (2004) suggested that EPCs have degrees of stemness including self-renewal, clonogenicity and differentiation capacity (Leri and Kajstura, 2005). Strictly speaking, however, progenitor cells have been distinguished from stem cells due to lack of self-renewing activity. Although the definition of EPCs is controversial, it is well accepted that EPCs have increased proliferative capacity and repairing ability (Young et al., 2005; Leeper et al., 2010), contributing to vascular recovery and angiogenesis.

CELLULAR CHARACTERISTICS OF EPCs

EPCs are thought to be differentiated from hemangioblasts along with hematopoietic stem cells, and located within the stem cell niche in bone marrow (Asahara and Kawamoto, 2004). There are also circulating EPC populations at low concentrations in the peripheral blood and the circulating EPC counts can be increased by stimulation under pathogenic condition such as ischemia, or by various factors including vascular endothelial growth factor (VEGF), stromal cell-derived factor-1 (SDF-1) or stem cell factor (SCF) (Urbich and Dimmeler, 2004; Holderfield and Hughes, 2008; Kim et al., 2011). Upon stimulation, bone marrow-derived EPCs migrate and incorporate into the vasculature and differentiate into mature endothelial cells (ECs). Through their proliferative and regenerative capacity, EPCs contribute to the restoration of normal vascular function and structure, as well as the growth of new vessels, which are also known as reendothelialization and neovascularization, resulting in enhanced angiogenesis (Urbich and Dimmeler, 2004; Tilki et al., 2009). Due to their unique characters of progressive differentiation, EPCs are usually described by cellular origin, the isolation methods and their surface markers. Several cell surface markers have been used for EPC identification, such as Flk-1 (VEGFR-2; KDR for human counterpart), Sca-1, CD34 and CD133. Unfortunately, there is no specific surface marker that definitely distinguishes EPCs. Peichev et al. (2000) characterized EPCs as VEGFR-2^+/$CD133^+$/$CD34^+$ cells, while Reyes et al. (2002) demonstrated that CD34/$CD133^+$/VEGFR-2^+ mesenchymal stem cells can differentiate to EPCs and generate into mature endothelial cells. Recently, Tian et al. (2009) employed Sca-1 as a bone marrow cell marker and used VE-cadherin and E-selectin as endothelial markers to identify EPCs. Currently, investigators use the approach that defines the hematopoietic lineage with endothelial cell character, along withthe distinct morphological cobblestone pattern and the capacity for tubular network formation in Matrigel for the characterizatIon of EPCs. The exact phenotype and the lineage of EPCs are not yet known, and most of the investigators demonstrate the functional characteristics of EPCs with their own characterization of isolated/cultured cells under the same experimental conditions.

EPC types

Although the markers for identification of EPC populations vary between studies, several investigators agreed that there are lineage and functional heterogeneities within the EPC population. There are at least two differEnt types of EPC3, which are early and late EPCs. Early EPCs are usually referred as the angiogenic EPC population obtained from short-term cultures of 4-7 days *in vitro*. Late EPCs, often called out-growth EPCs, have different growth patterns and are usually obtained from long term cultures of at least 2-4 weeks *in vitro* (Zhang et al., 2009). These two types of cells have several distinct aspects such as morphology, growth rate, cellular markers and involvement of certain diseases. Hur et al. (2004) showed that early EPCs have spindle-like shapes while late EPCs have a cobblestone shape. They also demonstrated that the number of early EPCs increased for 2 weeks after *in*

vitro culture followed by a gradual disappearance, but late EPCs appeared in 2 to 4 weeks and grew rapidly. Shantsila et al. (2008) focused on the differential distribution of cellular makers between early and late EPCs. Despite the challenges for defining specific markers for these cells with continual maturation and antigenic marker shifts, they suggested that early EPCs show CD14/CD45/KDRlow and then CD31/vWF, while late EPCs express CD34/KDR and CD31/vWF. They claimed that EPC subpopulations may display different angiogenic properties, as early EPCs have a relatively low proliferative capacity and a low ability to express mature endothelial proteins (Hur et al., 2004; Shantsila et al., 2008). Interestingly, it was reported that the number of late EPCs is higher in patients with coronary disease than normal subjects, whereas early EPCs show the opposite pattern, suggesting that the pathological roles of these two types of EPCs might be different (Hill et al., 2003; Guven et al., 2006).

Cellular function of EPCs

In normal physiology, ECs play important roles in regulating vessel constriction/relaxation, thrombosis, coagulation and adhesion of blood cells. Damage to ECs occur under pathological conditions or by xenobiotic exposure, leading to disruption of vascular homeostasis (Dimmeler and Zeiher, 2000; Verma and Anderson, 2002). As discussed above, EPCs have the capacity to proliferate, migrate, and differentiate into mature ECs to recover vascular function. Regeneration of ECs by incorporating EPCs into injured vessels is critical in terms of endothelium maintenance and restoration of normal EC function. Several clinical and experimental reports showed that EPCs have important roles in vascular regeneration. Takahashi and Yamanaka (2006) demonstrated that circulating mononuclear blood cells including EPCs can differentiate to an EC phenotype and can incorporate into capillary vessels. Shi et al. (1998) showed that bone marrow transplantation to adult dogs resulted in the formation of a new endothelial lineage in aorta after graft lesion by $CD34^+$ cells of donor origin. Using an ischemic hindlimb model, Ikenaga et al. (2001) proved that bone marrow transplantation enhanced angiogenesis, and Chen et al. (2009) found that transplantation of *ex vivo* expanded EPCs improved neovascularization in a porcine model of chronic myocardial ischemia, improving vascular density in the damaged area. The vascular regenerative capacity of EPCs has been also demonstrated in clinical trials, where bone marrow cells containing EPCs were transplanted to human patients with coronary artery diseases (Tse et al., 2007) or infarcted myocardium (Assmus et al., 2002; Strauer et al., 2002). Conclusively, *ex vivo* expanded or *in vivo* transplanted EPCs have shown their regenerative and repairing activity in damaged endothelial lesions through their adhesive, proliferative and migratory abilities as well as by their capacity for tubular capillary network formation. The therapeutic potential of EPCs are promising and many investigators are trying to identify the regulatory mechanisms underlying EPC function prior to further application of EPC in the clinical.

EPCs in cardiovascular diseases

Numerous studies have supported the notion that EPCs are deregulated by cardiovascular risk factors or in cardiovascular diseases through clinical data. The number of EPCs both in circulation and in the bone marrow niche is decreased, and the functional capacities of circulating EPCs or *ex vivo* expanded EPCs are significantly impaired by cardiovascular events (Hill et al., 2003; Werner et al., 2005; Tilki et al., 2009). In hypertensive patients, the circulating number and migratory capacity of EPCs have decreased (Vasa et al., 2001; Huang et al., 2010), and the role of angiotensin II in impaired EPC function under hypertension has been suggested through its effect on EPC senescence in addition to reduced proliferation in *in vitro* and *in vivo* studies (Imanishi et al., 2005; Endtmann et al., 2011). Abnormal lipid metabolism including cholesterol, lipoprotein and triglycerides are related to impaired function and decreased numbers of circulating EPCs. Oxidized low density lipoprotein (oxLDL) exposure to culture EPCs results in senescence, apoptosis as well as decreasing EPC differentiation and numbers (Imanishi et al., 2004; Wu et al., 2009; Tie et al., 2010). We will discuss in more detail oxLDL-induced EPC dysfunction in migratory capacity later. High serum cholesterol levels are inversely correlated to the number and functional properties of EPCs in patients with coronary artery diseases (CADs) (Vasa et al., 2001; Hill et al., 2003). Diabetes mellitus is other representative cardiovascular risk factor associated with decreased number and function of EPCs, as we will focus on the diabetic EPC impairment in this article. Endogenous mediators which enhance the development of cardiovascular problems, such as homocysteine or C-reactive protein (CRP), can also affect EPC function. Chen et al. (2004) showed that homocysteine exposure reduced the number of EPCs isolated from the peripheral circulation and inhibited EPC function such as proliferation, adhesion and migration. Alam et al. (2009) proved that the level of homocysteine is inversely correlated to circulating EPC

number in patients with stroke. The levels of CRP were related with the number of circulating EPCs in patients with unstable angina (George et al., 2004), and Fujii et al. (2006) showed that CRP alters antioxidant defenses, inhibits proliferation and promotes apoptosis in EPCs. Besides the relationship with cardiovascular risk factors, EPC dysregulation of number and function is seen in various cardiovascular diseases. Circulating EPC levels are significantly decreased in patients after stroke (Taguchi et al., 2004; Ghani et al., 2005) and in those with atherosclerosis (Xiao et al., 2007) or rheumatic diseases (Westerweel and Verhaar, 2009). The degree of decreased number of EPCs in patients with heart failure is found to be related to the stage of heart failure, with relatively higher numbers in the early stage and progressively lower numbers in severe heart failure (Valgimigli et al., 2004). A strong correlation with EPC dysfunction and CAD, including ischemic heart diseases has been reported in several reports, and Heeschen et al. found that isolated bone marrow cells from patients with chronic CAD have reduced neovascularization capacity and impaired ability to improve tissue (Heeschen et al., 2004). Interestingly, there is a close relationship between circulating EPC number and their functions (Tousoulis et al., 2008). Recently, studies have shown that circulating EPC number could be a predictor for defective vascular regenerative capacity in clinical conditions. Schmidt-Lucke et al. (2005) suggested that decreased number of EPCs which express CD34 and VEGFR-2 is an independent risk biomarker for cardiovascular events. Fadini et al. (2006a, 2006b) reported that the number and function of EPCs can be used as a determinant marker of the severity of vascular issues after diabetes or subclinical atherosclerosis.

EPC DYSFUNCTION UNDER DIABETIC CONDITIONS

Abnormality of EPCs has been related to various cardiovascular symptoms and severe vascular complications are known to be major concerns in diabetes-associated cardiovascular complications (Tepper et al., 2002; Loomans et al., 2004, 2005; Fadini et al., 2005). Numerous studies demonstrated that endothelial dysfunction is the key initiator that precedes development of vascular problems in diabetes (Dimmeler and Zeiher, 2000; Verma and Anderson, 2002). Impaired endothelial regeneration and retarded angiogenesis contribute to the progression of diabetic vascular complications, and therefore the roles of EPCs have been raised as one of the major concerns (Liew et al., 2008; Jarajapu and Grant, 2010). Until now, most of the studies related to EPC function in diabetes primarily focused on early EPCs. The decreased number and function of EPCs were found to be associated with diabetes-related complications (Loomans et al., 2004; Fadini et al., 2006b). Recently, Chen et al. (2007) demonstrated that early and late EPCs can be impaired by high glucose through nitric oxide signaling. In this section, we will focus on the clinical evidencefor EPC dysfunction in diabetic patients.

Decreased EPC counts in diabetes

EPC abnormality in the clinical can be seen in several situations. First, the circulating number of EPCs in diabetic patients is significantly reduced compared to non-diabetic controls. This reduction has been reported in both types of diabetes, type 1 and type 2 diabetes (Tepper et al., 2002; Loomans et al., 2004; Fadini et al., 2005; Reinhard et al., 2010). Loomans et al. (2004) isolated and cultured peripheral blood mononuclear cells (PBMCs) from type 1 diabetic patients and healthy controls. The differentiated number of EPCs from patients with type 1 diabetes was decreased by 44% compared to the control group. Interestingly, the severity of EPC reduction is well correlated to the levels of HbA1C, which is a representative marker for diabetic conditions (Loomans et al., 2004). Reduction of EPC number was also found in patients with type 2 diabetes (Tepper et al., 2002; Fadini et al., 2005). The number of circulating EPCs was analyzed using a EPC/CPC ratio, which represents the fraction of EPCs among all circulating progenitor cells. The number of *ex vivo* expanded EPCs isolated from patients with type 2 diabetes was decreased, and the reduced number of EPCs was increased after multifactorial treatment which induced glycemic control and total cholesterol improvement (Reinhard et al., 2010).

Functional impairment in diabetic EPCs

Besides the reduction of circulating EPCs, several clinical studies have supported the role of functional damage to EPCs under diabetic conditions (Tepper et al., 2002; Loomans et al., 2004; Gallagher et al., 2007; Shantsila et al., 2007; Liew et al., 2008; Jarajapu and Grant, 2010). EPCs isolated from diabetic patients showed impaired abilities in proliferation, adhesion and incorporation into blood vessels, resulting in angiogenic dysfunction. Tepper et al. (2002) demonstrated that diabetic EPCs had a poor proliferation ability compared to non-diabetic EPCs, when they were isolated and cultured for 7 days. They also reported that EPC adhesion ability is significantly impaired under type 2 diabetic conditions to matrix molecules

as well as to HUVEC monolayers activated with TNF-α, which is a crucial character during new vessel growth. The incorporation of EPCs from diabetes into vascular structures was significantly lower than that of EPCs from healthy controls, suggesting that overall angiogenic function of the EPC was impaired in diabetic patients (Tepper et al., 2003; Shantsila et al., 2007). Moreover, EPCs isolated from diabetic patients showed functional impairment which correlated to the severity of peripheral artery diseases, suggesting the pathogenic role of EPC dysregulation in diabetic vasculopathy (Fadini et al., 2006b). Using an *in vivo* animal model, Sorrentino et al. (2007) transplanted EPCs from healthy subjects or type 2 diabetic patients into denuded carotid arteries in nude mice. The damaged endothelial layer of arteries was not repaired by diabetic EPC transplantation, whereas significant improvement was found in groups transplanted with normal EPCs.

Reduced responses to stimuli by diabetic EPCs

Diabetic EPCs showed impaired responses to activating stimuli. When peripheral tissue is under a certain stimulus such as ischemic injury, EPCs can migrate into the tissue then differentiate to ECs in order to create a new vessel. However, diabetic EPCs have impaired ability in response to this stimulus. Capla et al. (2007) demonstrated that type 2 diabetic EPCs had impaired functions under hypoxic conditions. But, there was no significant functional difference between type 2 diabetic EPCs and non-diabetic EPCs during normoxia. On the other hand, diabetic EPCs showed significantly impaired adhesion, migration and proliferation under hypoxia. Similarly, Segal et al. (2006) demonstrated that EPCs isolated from diabetic patients had a decreased response to SDF-1, a representative recruiting stimulus for EPC migration, suggesting that diabetic EPCs have an impaired ability in response to stimulus leading to decreased angiogenesis and delayed vascular regeneration.

Paracrine manners in diabetic condition

EPCs contribute to neovascularization not only by integration but also in a paracrine manner where EPCs secrete several factors to the surrounding environment. Interestingly, this secretory ability of EPCs is reported to be abnormal in the diabetic condition. Conditioned media from type 1 diabetic EPCs resulted in angiogenesis impairment compared to either conditioned media from non-diabetic EPCs or non-conditioned media from diabetic EPCs (Loomans et al., 2004). This finding suggests that diabetic EPCs lose their angiogenic secretory ability and may secrete other anti-angiogenic factors.

SUGGESTED MECHANISMS FOR DIABETIC EPC DYSFUNCTION

Elucidation of the underlying mechanisms for diabetic EPC dysfunction has been of great interest in the pharmaceutical and medicinal fields to address the possible use of EPCs in diabetes-associated cardiovascular symptoms. Several mechanisms have been suggested, based on evidence from *in vitro* bioassays as well as *in vivo* animal models (Fig. 1). Here we will discuss the mechanisms responsible for the functional damage in diabetic EPCs, especially for their migratory activity which affects EPC recruitment and incorporation into damaged vessels.

Reactive oxygen species

Excessive generation of reactive oxygen species (ROS) has been explained as one of the mediators of diabetes-associated cardiovascular complications. Many studies demonstrated that ROS production in diabetic EPCs is also significantly higher than normal EPCs. In EPCs isolated from type 2 diabetic mice, the levels of antioxidant enzymes such as MnSOD are significantly decreased (Marrotte et al., 2010) and overexpression of MnSOD effectively reversed diabetic EPC

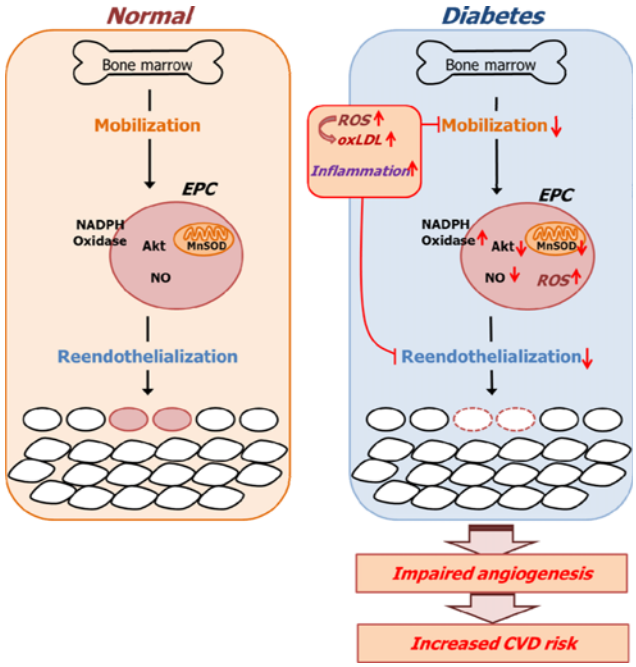

Fig. 1. Suggested mechanisms underlying diabetic EPC dysfunction. EPC, endothelial progenitor cells; CVD, cardiovascular disease; NO, nitric oxide; ROS, reactive oxygen species; oxLDL, oxidized low density lipoprotein; MnSOD, manganese superoxide dismutase

dysfunction including tube formation and migration. They also observed that improved *in vivo* wound healing capacity is significantly improved by cell transplantation with normal EPCs to diabetic mice, and this improvement was mimicked by transplantation with MnSOD-overexpressed diabetic EPCs. Several studies also demonstrated that ROS generating systems such as NADPH oxidases are excessively activated in diabetic EPCs (Sorrentino et al., 2007; Werner et al., 2007). When they inhibit NADPH oxidase through overexpression of dominant negative subunits or siRNAs, or by decreasing excessive oxidative stress using antioxidants, impaired EPC functions such as migration, adhesion, proliferation and *in vivo* reendothelialization were improved (Sorrentino et al., 2007; Ceradini et al., 2008). This supports the notion that increased ROS generation contributes to diabetic EPC dysfunction.

Oxidized LDL

Under diabetic condition, increased oxidative stress results in excessive production of oxLDL (Hamed et al., 2011). High levels of circulating oxLDL are known to contribute to cardiovascular symptoms such as CAD in type 2 diabetic patients (Shimada et al., 2004). oxLDL is one of the factors that was associated with survival and function of EPCs (Imanishi et al., 2004; Ma et al., 2006; Tie et al., 2010). Although oxLDL did not affect SDF-1α-induced expression of CXC receptor-4 (CXCR-4) and was associated with EPCs migration, oxLDL decreased Akt phosphorylation and eNOS expression in normal EPCs (Ma et al., 2006; Hamed et al., 2010). When EPCs were exposed to hyperglycemia, however, the level of CXCR-4 was modulated. Simultaneous exposure of normal EPCs to hyperglycemia and oxLDL decreased expressions of CXCR-4 and eNOS as well as reduced eNOS and Akt phosphorylation. The alteration of these cellular signaling pathways resulted in impaired EPCs migration (Hamed et al., 2010). Furthermore, oxLDL significantly decreased production of nitric oxide (NO), which is an important mediator for EPC function as discussed below, leading to reduced survival of EPCs (Hamed et al., 2010). oxLDL-associated NO decreases and Akt pathway impairment are known to induce EPC apoptosis in addition to disrupting EPC adhesion, migration and tube formation (Ma et al., 2006). Therefore, increased generation of oxLDL in diabetic patients is suggested as one of the potential mechanisms underlying diabetic EPC dysfunction.

Defective nitric oxide pathway

The NO and cyclic GMP (cGMP) axis is one of the representative regulatory pathways in normal endothelial function (Moncada and Higgs, 2006). Likewise, EPCs require NO-cGMP signaling for proper function including migration, and NO signaling in EPCs is damaged by diabetes (Irie et al., 2005; Segal et al., 2006; Hamed et al., 2011). Cell migration is regulated by the Ena/VASP protein family which plays a crucial role in promoting actin filament elongation at the leading edge by forming an active molecular motor complex that propels motility. Ena/VASP proteins are substrates for phosphorylation by cyclic-nucleotide-dependent protein kinase at a number of sites and it is known that they are regulated by protein kinase G (PKG) which is activated by cGMP generated through NO-stimulated soluble guanylate cyclase (sGC) activation (Butt et al., 1994; Li Calzi et al., 2008). Cell movement requires a coordinated cycle of adhesion and detachment, and NO-induced regulation of cytoskeleton might be subject to rapid changes that allow for cycles of lamellipodial extension and retraction (Lindsay et al., 2007). In EPCs isolated from diabetic patients showed significantly reduced NO bioavailability and impaired endothelial nitric oxide synthase (eNOS) activity (Segal et al., 2006; Hamed et al., 2011). Moreover, as discussed earlier, increased generation of reactive oxygen species, including superoxide anion, decreased NO concentrations and NO bioavailability in EPCs under diabetic conditions (Thum et al., 2007b). Reduced NO concentration is not sufficient to regulate VASP phosphorylation and actin polymerization for cell migration, contributing to defective migratory activity in diabetic EPCs. Segal et al. (2006) demonstrated that EPCs isolated from diabetic patients showed impaired migration to SDF-1 stimulation through defective cell deformability and they proved that exogenous NO corrected impaired cytoskeletal arrangement and migratory response to SDF-1.

Impaired mobilization signaling

As we discussed for oxLDL, NO bioavailability could modulate the mobilization of EPCs into the circulation. EPC mobilization from the bone marrow depends on the activation of eNOS in the presence of several mobilizing factors (Leone et al., 2009). Substances that increase NO bioavailability such as growth hormone (GH) and insulin growth factor-1 (IGF-1), enhance the number of circulating EPCs (Thum et al., 2007a). In contrast, substances that impair NO bioavailability like asymmetric dimethylarginine (ADMA), are known to decrease circulating EPC populations. Decreased NO bioavailability results in impaired EPC recruitment to the circulation under diabetic condition. In addition to the role of NO, several mediators cooper-

ate to induce EPC mobilization. In normal conditions, mobilization is regulated by growth factors such as SDF-1, G-CSF, fibroblast growth factor (FGF) and VEGF with the involvement of matrix metalloproteinases such as MMP-2 and MMP-9, cathepsin-G and elastase. However, the recruiting signals due to vascular injury are weaker in diabetes, resulting in decreased mobilization of EPCs into the circulation (Jarajapu and Grant, 2010). Disrupted regulation of the SDF-1/CXCR-4 axis is known to contribute to impaired EPC mobilization (Jujo et al., 2010). Egan et al. (2008) observed that circulating EPCs were significantly reduced in type 2 diabetic patients and reduced EPC levels were well correlated with a decreased expression in CXCR-4. In addition, the levels of putative CXCR-4 positive cells were further decreased in patients with diabetic cardiovascular complications suggesting that impaired CXCR-4 expression play key roles in diabetic EPC mobilization and function (Egan et al., 2008). Fadini et al. demonstrated that the diabetic defect in the release of EPCs is reversed by glucose normalization, implying that the mobilization mechanisms are sensitive to chronic hyperglycemic conditions (Fadini et al., 2007). Interestingly, Busik et al. (2009) showed that the numbers of EPCs within the bone marrow is increased while circulating EPCs are significantly decreased in a rat model of type 2 diabetes, confirming that damaged migratory activity may be the main cause for the reduced levels of circulating EPCs.

Inflammatory mediators

Diabetes is known to be closely linked to chronic inflammation (Schuster, 2010). Notably, inflammation affects both EPC number and function, and EPCs react in two different ways to an inflammatory environment. In low levels of inflammatory cytokines, the number and function of EPCs are positively regulated. Low inflammatory cytokine concentrations help recruiting and adhering of EPCs to the injured area, and the circulating numbers of EPCs are increased (Rabelink et al., 2004; Tousoulis et al., 2008). However, in a severe and chronic inflammatory environment such as diabetes, EPC functions including mobilization, adhesive ability and proliferation are impaired, and the numbers of EPCs are reduced leading to retarded angiogenesis. Inflammatory cytokines including TNF-α are increased in hyperglycemic conditions, it induces EPC apoptosis, insulin-resistance and reduction of AKT phosphorylation, leading to a reduction in the number of EPCs. Inhibition of NF-B, which regulates TNF-α and other inflammatory mediators, reversed EPC apoptosis and insulin-resistance thereby improving impaired EPC migration (Desouza et al., 2011).

RESTORATION OF DIABETIC EPC FUNCTION

In an attempt to address the therapeutic potential of EPCs for diabetes-associated cardiovascular complications, recent studies demonstrated that diabetic EPC dysfunction could be modulated by pharmacological or genetic intervention. As summarized in Table I, several treatments affected molecular pathways including eNOS activity, CXCR-4 expression and heme-oxygenase-1 (HO-1) expression in EPCs, resulting in

Table I. Genetic and pharmacological intervention for diabetic EPC recovery

Therapy	Gene or Drug	Target	Subject	Effects on EPC	References
Genetic	MnSOD	MnSOD	Mouse	↑Angiogenesis, ↑Wound healing	Marrotte et al. (2010)
Genetic	shh	shh	Mouse	↑Wound healing, ↑Proliferation, ↑Migration, ↑Adhesion, ↑Tube formation	Asai et al. (2006)
Pharmacological	D-4F	Up-regulation of HO-1, eNOS	Rat	↑ HO-1 activity, ↑eNOS level, ↑Proliferation	Peterson et al. (2007)
Pharmacological	Rosiglitazone	PPAR-γ	Human	↑Migration, ↑EPCs number	Pistrosch et al. (2005)
Pharmacological	Pioglitazone	PPAR-γ	Human	↑Adhesion, ↑ CXCR-4 expression	Ruiz et al. (2009)
Pharmacological	UAG (Unacylated Ghrelin)	Ghrelin pathways	Human, Mouse	↑Mobilization, ↑Tube formation, ↑eNOS activity	Togliatto et al. (2010)
Pharmacological	Nitropravastatin (NO-releasing pravastatin derivative)	HMG-CoA reductase, NO	Mouse	↑EPCs number, ↑Migration	Emanueli et al. (2007)
Pharmacological	Olmesartan, Irbesartan	Angiotensin II Rc	Human	↑EPCs number	Bahlmann et al. (2005)

functional recovery of mobilization, adhesion, tube formation and proliferation. These alterations ultimately contributed to *in vivo* improvement of cardiovascular complications like angiogenesis and wound healing in the diabetic condition, supporting the potential use of EPC cell therapy against diabetic vascular problems.

CONCLUDING REMARK

A variety of signaling molecules and pathways are involved in diabetic EPC dysfunction. Besides impaired intracellular homeostasis including excessive NADPH oxidase activation, decreased NO pathway function and defective cytoskeleton regulation inside diabetic EPCs, complicated pathophysiological diabetic environments such as increased oxidative stress, inflammation and activating/inhibiting signaling mediators may contribute to the dysregulation of diabetic EPCs. With an overall understanding of the mechanisms underlying diabetic EPC dysfunction, potential for the use of EPCs in the clinical setting can be considered. Moreover, strategies for the development of chemicals or drugs that improve EPC function may be promising for the prevention and/or treatment of diabetes-associated cardiovascular symptoms. It may not be easy to develop the optimal strategy for effective EPC therapy or to develop drug candidates for EPC functional recovery, however, more clinical and laboratory evidence will help us to understand EPCs better, leading us to increase their therapeutic potential in clinical trials.

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