Effects of Cardiovascular Risk Factors on Endothelial Progenitor Cell

Po-Hsun Huang,[1,3,5] Jaw-Wen Chen[1,2,4,5] and Shing-Jong Lin[1,2,3,5]

Atherosclerosis is a systemic inflammatory disease of arterial wall and initiated by endothelial damage. The integrity and functional activity of endothelial monolayer play an important role in atherogenesis. The extent of endothelial injury may represent a balance between the magnitude of injury and the capacity for repair. Traditional view suggested endothelium integrity is maintained by neighboring mature endothelial cells which migrate and proliferate to restore the injured endothelial cells. However, a series of clinical and basic studies prompted by the discovery of bone marrow-derived endothelial progenitor cells (EPCs) have demonstrated that the injured endothelial monolayer may be regenerated partly by circulating EPCs. These circulating EPCs are mobilized endogenously triggered by tissue ischemia or exogenously by cytokine stimulation. Clinical studies demonstrated that levels of circulating EPCs are associated with vascular endothelial function and cardiovascular risk factors, and help to identify patients at increased cardiovascular risk. Reduced levels of circulating EPCs independently predict atherosclerotic disease progression and development of cardiovascular events. Therefore, a better understanding of the relation between EPCs and atherosclerosis would provide additional insight into the pathogenesis of cardiovascular disease and create novel therapeutic strategies. Here, we will make a brief review to clarify the effects of cardiovascular risk factors on circulating EPCs.

Key Words: Atherosclerosis ● endothelial function ● endothelial progenitor cell

1. INTRODUCTION

The last decade has seen a substantial and growing interest in the field of regenerative biology, with particular emphasis on the use of isolated or purified stem and progenitor cells to restore structure and function to damaged organs. Circulating endothelial progenitor cells (EPC) were first discovered in 1997 by Asahara et al., who identified in the adult human peripheral blood a population of CD34 or kinase insert domain receptor (KDR)-positive cells which have been studied as a potential cell source that contributes to neovascularization via postnatal vasculogenesis.[1] At the time, this notion challenged the established belief that *de novo* formation of new blood vessels occurs only in the yolk sac mesoderm during embryonic development. It was soon recognized that EPCs derive from the bone marrow and can be mobilized into the peripheral circulation in response to many stimuli, including tissue ischemia and vascular damage, through the release of growth factors and cytokines.[2]

It is well-known that the integrity and functional activity of the endothelial monolayer play an important role in atherogenesis.[3] Endothelial dysfunction and injury are considered to be the first steps in atherogenesis. The traditional view suggests that endothelium integrity is maintained by neighboring mature endothe-

lial cells which migrate and proliferate to restore the injured endothelial cells. However, a series of clinical and basic studies have provided new evidence that the injured endothelial monolayer is regenerated by circulating EPCs.[4] The circulating EPC number has also been reported to inversely correlate with the presence of risk factors of coronary artery disease (CAD).[5-7] An altered status of circulating EPCs represents a marker of endothelial dysfunction and reflects on vascular health.[8] The capacity of circulating EPCs to repair vascular injury suggests that they may play a pivotal role in maintaining homeostasis of the endothelium. Therefore, we have undertaken to generate a summary review and focus on the differential effects of various cardiovascular risk factors on EPCs.

2. EPC AND ENDOTHELIAL FUNCTION

A key early event in atherosclerosis is endothelial cell dysfunction, which is precipitated by several factors including metabolic syndrome, diabetes, hypertension, dyslipidemia, chronic kidney disease (CKD), and smoking, all features of cardiovascular risk factors. Intact endothelium and maintenance of endothelial integrity play a critical role in preventing the development of atherosclerotic vascular disease.[9] Once in the bloodstream, EPCs constitute a pool of circulating cells able to form a cellular patch at sites of endothelial denudation, in order to restore endothelial integrity.[10] This action has been demonstrated in several experimental models and is accomplished through the expression of surface adhesion molecules and chemokine receptors.[11,12] Cell tracking systems employing green fluorescence protein positive bone marrow chimeric animals confirmed that bone marrow-derived endothelial cells physically take part in new vessel formation.[13]

Based on previous studies, circulating EPC was suggested to play two major roles in the cardiovascular system: endothelial healing and neovascularization. With this functional responsibility, it is easy to anticipate that a reduction in circulating EPCs would translate into an inability to maintain a healthy endothelium and impaired post-ischemic angiogenesis, favoring development and progression of cardiovascular diseases.[14]

Since these important findings, an enormous amount of research has been undertaken into EPCs; however, in an attempt to collate and interpret these results, a major limiting factor is that no simple definition of EPCs exists at the present time, and various methods used to defined EPC have been reported. This pertains to the unresolved issue of how EPCs should best be defined. Currently, the first and most accepted method of classification of EPC is based on expression of cell-surface antigens, typically using flow cytometry to quantify relevant populations. EPCs can be quantified by the number of circulating CD34$^+$/vascular endothelial growth factor receptors (VEGFR)-2$^+$ or CD34$^+$/VEGFR-2$^+$/ CD133$^+$ cells or by the number of colonies of adherent cells that can be obtained from circulating mononuclear cells that express mature endothelial cell markers. Clinical studies have shown that the level of circulating EPCs is directly related to measures of peripheral and coronary endothelial function.[15-17] A significant reduction of circulating EPCs is also associated with the earliest signs of atherosclerotic remodeling, such as increased carotid intima-media thickness and even hypertension-related hypertrophy.[18,19] Remarkably, nearly all classical cardiovascular risk factors have been associated with a significant reduction or dysfunction of EPCs. Therefore, EPC alterations are now considered to be a major mechanism by which risk factors translate into impaired cardiovascular homeostasis. The reversal of EPC dysfunction could therefore potentially prevent the progression of cardiovascular and vascular disease.

3. EFFECTS OF CARDIOVASCULAR RISK FACTORS ON EPC

3-1. Metabolic syndrome and EPC

Metabolic syndrome comprises a cluster of abnormalities, with insulin resistance and adiposity as central features. Furthermore, metabolic syndrome confers a two- to fourfold increased risk for cardiovascular disease and fivefold increased risk of diabetes.[20] Westerweel et al. showed that circulating CD34$^+$KDR$^+$ EPC levels were reduced by nearly 40% in obese men with metabolic syndrome compared to nonobese men.[21] Jialal and colleagues reported that metabolic syndrome subjects without diabetes or cardiovascular disease have a decreased EPC number and impaired functionality as com-

pared to control subjects.[22] In addition to the reduction in numbers, they showed that there was significant impaired clonogenic capacity and an impaired capacity to incorporate into tubule structures. These findings suggest the defects in EPCs manifest early in metabolic syndrome prior to the development of diabetes or cardiovascular disease. Satoh et al. have reported increased EPC number in CAD patients with metabolic syndrome and without metabolic syndrome.[23] Interestingly, they also showed increased oxidative DNA damage, decreased telomerase activity, and decreased telomere length, a marker of increased senescence in EPCs of CAD patients with metabolic syndrome than the CAD patients without metabolic syndrome. Patients with nonalcoholic fatty liver disease, a hepatic manifestation of the metabolic syndrome, were also demonstrated to have decreased circulating EPC number and functions than those without nonalcoholic fatty liver disease.[24] These reports indicate EPC alterations in the bloodstream in metabolic syndrome may be one of the mechanisms that can help to explain atherosclerotic disease progression and enhanced cardiovascular risk in patients with metabolic syndrome.

3-2. Diabetes and EPC

Diabetes mellitus has reached epidemic proportions worldwide and is associated with a large economic burden, an increased risk of cardiovascular disease, poor outcomes as a result of vascular occlusion, and premature mortality. Diabetic patients frequently develop micro- or macro-vascular abnormalities such as retinopathy, nephropathy, and accelerated atherosclerosis. The mechanisms that underlie this reduced count and impaired functionality are poorly understood. Considerable evidence has indicated that vascular endothelial function is impaired in diabetes patients. It is evident that a decrease in nitric oxide production by endothelial nitric oxide synthase (eNOS) plays a critical role in the development and progression of atherosclerosis in diabetes. Clinical studies have reported that EPCs are markedly reduced in patients with either type 1 or type 2 diabetes, and EPCs from diabetic patients also show reduced capacity to induce angiogenesis *in vitro*.[25] Likewise, impaired post-ischemic EPC mobilization in diabetic animals has been demonstrated previously.[26,27] These defects in EPC functions and behavior may under-

lie some of the vascular complications associated with diabetes, such as endothelial dysfunction, that predispose a diabetic patient to diffuse atherosclerosis and impaired neovascularization after ischemic events. There is general agreement that hyperglycemia and diabetes lead to impaired nitric oxide production and activity. Our previous study further demonstrated that long-term exposure to high glucose might enhance cellular senescence and decrease cell numbers and functional competencies of EPCs via nitric oxide-related mechanisms.[28] Moreover, the results of several studies have demonstrated that hyperglycemia or oxidized low-density lipoprotein (oxLDL) can reduce both the EPC count and impair EPC migration and proliferation by exerting a deleterious effect on the phosphatidylinositol-3 kinase/protein kinase B (PKB)/Akt/eNOS/ nitric oxide signaling cascade.[29,30] These findings provide potential therapeutic targets for hyperglycemia-related vascular complications in diabetic patients.

3-3. Hypercholesterolemia and EPC

Hypercholesterolemia is a major risk factor for atherosclerosis and CAD. It is commonly understood that endothelial dysfunction elicited by hypercholesterolemia plays a pivotal role in the development of atherosclerosis.[31] Impaired endothelial function ultimately represents a balance between the magnitude of injury and the capacity for repair. A variety of evidence suggests that circulating EPCs constitute one aspect of this repair process. Clinical studies have documented that hypercholesterolemia can decrease EPC number and activity.[6] Tie and coworkers showed that oxidized low-density lipoprotein (oxLDL) is taken up by EPCs in a receptor-dependent manner wherein it generates oxidative stress.[32] Additionally, oxLDL-induced oxidative stress can lead to cell necrosis or apoptosis.[33] These results suggest that oxLDL may impair EPC survival, a circumstance that would impair their participation in postischemic vascular repair. Given the well-established role of EPCs in participating in neovascularization and re-endothelialization, these findings may establish a novel pathophysiological mechanism of hypercholesterolemia. Hypercholesterolemia not only impairs endothelial cells directly, but also attenuates the EPC number and function at the same time. Thus hypercholesterolemia may influence the endothelial repair process and disturb the balance

between the magnitude of injury and the capacity for repair, which leads to endothelial dysfunction, and promotes the progression of CAD.

Moreover, high-density lipoprotein (HDL) was previously suggested to protect against the progression of atherosclerosis because serum HDL levels could be reduced in patients with CAD, and reduced HDL level might be an independent risk factor for cardiovascular diseases.[34] However, clinical evidence raised the disagreement regarding whether HDL could exert a protective effect, especially in healthy subjects.[35] Recently, Huang et al. indicated the differential effects of native HDL on EPCs in the presence and absence of oxLDL, which suggests the conditional direct protective effects of HDL in the presence of oxLDL.[36] In the absence of oxLDL, native HDL at low concentrations promoted EPC function by activating eNOS mechanisms, whereas HDL at normal to high physiological concentrations enhanced EPC senescence and impaired EPC function. These novel findings may provide the mechanistic evidence of concentration-related biphasic effects of HDL on EPC.[36]

3-4. Hypertension and EPC

There is no doubt that hypertension is one of the most well-known cardiovascular risk factors for target organ damage and cardiovascular events. With regard to hypertension, it has been shown that patients with CAD have a reduced level and migratory capacity of EPCs, and the latter is mainly influenced by hypertension. Furthermore, Kim et al. had indicated that the EPC count was reduced in the peripheral bloodstream in non-dipper hypertensive patients.[37] Moreover, even in the pre-hypertension stage, MacEneaney et al. had indicated that the ability of EPCs to form colonies is impaired in pre-hypertensive adults with systolic blood pressure greater than 130 mmHg, although pre-hypertension is not associated with diminished EPC migratory capacity or increased apoptotic susceptibility.[38] Our recent data further showed that essential hypertensive patients with electrocardiographic left ventricular hypertrophy (LVH) evidence have decreased circulating EPC numbers and adhesive function compared to those patients without LVH evidence.[19] These findings may explain the pathogenetic processes that link hypertension and endothelial injury in cardiovascular disease.

3-5. Chronic kidney disease and EPC

CKD is a worldwide public health problem with an increasing incidence and prevalence. Renal insufficiency has been demonstrated as an independent risk factor for all-cause mortality, as well as adverse cardiovascular disease outcomes including myocardial infarction, stroke, and progression of heart failure.[39] Progression of CKD is associated with decreased endothelial function, increased prevalence of atherosclerosis, systemic inflammation, and enhanced reactive oxygen species production, all of which have been associated with cardiovascular risk and mortality.[40] In a cross-sectional, prospective study involving 50 patients with varying degrees of CKD, Krenning et al. indicated that EPC number and function decrease with advancing CKD, which may hamper physiological vascular repair and can add to the increased risk for cardiovascular diseases observed in CKD patients.[41] Of note, our recent studies had demonstrated that hypertensive patients with nephropathy have decreased circulating EPC numbers.[42] An increased circulating endothelial microparticle to EPC ratio is significantly associated with subsequent decline in renal function estimated by the glomerular filtration rate in hypertensive patients.[43] Additionally, decreased circulating EPC level was also observed in patients who develop contrast-induced nephropathy,[44] which is well-recognized as a serious clinical problem connected to the use of iodinated contrast media and is associated with cardiovascular risk.[45] These data clearly indicate endothelial damage with reduced vascular repair capacity may contribute to deterioration of renal function in patients with cardiovascular risk factors or CAD.

3-6. Smoking and EPC

Epidemiological studies have indicated that cigarette smoking is a leading cause of preventable cardiovascular death. Cigarette smoking accounts for almost 50% of coronary events, and the risk of myocardial infarction or stroke decreases by 50% within the first 2 years after smoking cessation.[46] Previous reports demonstrated that chronic smokers have endothelial dysfunction,[47] and endothelial reactivity is rapidly restored after smoking cessation.[48] Several studies mentioned the relationship between smoking and circulating EPC. Kondo et al. indicated the number of circulating EPCs was reduced in chronic smokers. Interesting, smoking

cessation led to a rapid restoration of EPC levels.[49] Furthermore, the recovery of circulating EPC levels was greater in light smokers than in heavy smokers.[50] Decreased number of circulating EPCs would make smokers susceptible to cardiovascular disease, and even a short-duration cessation of smoking may be an effective means of reducing cardiovascular risk.

In fact, smoking is a well-known factor inhibiting the release of physiological amounts of nitric oxide produced by endothelial nitric oxide synthase, and a recent study has demonstrated that eNOS is important for EPC mobilization from bone marrow.[50] Second, change in EPC levels associated with smoking status is possibly related to the fact that chronic smokers have impaired nitric oxide bioavailability.[51] Taken together, impaired endothelial function in smokers may use EPCs to maintain endothelial function, and that the increase in circulating EPCs after smoking cessation may be the result of a decreased number of injured vessels after cessation.

The effects of cardiovascular risk factors on EPC are briefly summarized in the Table 1.

Table 1. Effects of cardiovascular risk factors on endothelial progenitor cell from clinical evidence

Cardiovascular risk factor vs. EPC	EPC number	EPC function	Comments	Reference
Metabolic syndrome	↓	↓	Circulating EPC levels are reduced in obese men with metabolic syndrome compared to nonobese men. Metabolic syndrome subjects without diabetes or cardiovascular disease have decreased EPC number and impaired functionality as compared to control subjects.	21-24
Diabetes	↓	↓	Decreased EPC number and function in Type 1 and Type 2 diabetes. Long-term exposure to high glucose might enhance cellular senescence and decrease cell numbers and functional competencies of EPCs via nitric oxide-related mechanisms.	25-29
Hypercholesterolemia (ox-LDL)	↓	↓	Hypercholesterolemia can decrease EPC number and activity.	6,32,33
HDL	↑ (with oxLDL)	↑ (with oxLDL)	HDL protects EPCs from the injury of oxLDL in a dose-dependent fashion.	36
	Biphasic effects (without oxLDL)	Biphasic effects (without oxLDL)	In the absence of oxLDL, native HDL at low concentrations promotes EPC function, whereas HDL at normal to high physiological concentrations enhances EPC senescence and impaired EPC function.	
Hypertension	↓	↓	EPC count is reduced in the peripheral bloodstream in non-dipper hypertensive patients. In prehypertension stage, the ability of EPCs to form colonies was shown to be impaired in prehypertensive adults. Essential hypertensive patients with electrocardiographic LVH evidence have decreased circulating EPC numbers and adhesive function compared to those without LVH evidence.	19,37,38
Chronic kidney disease	↓	↓	EPC number and function decrease with advancing CKD. Hypertensive patients with nephropathy have decreased numbers of circulating EPCs, and an increased circulating endothelial microparticle to EPC ratio is significantly associated with subsequent decline in renal function in hypertensive patients.	42-44
Smoking	↓	↓	Circulating EPCs number is reduced in chronic smokers, and smoking cessation leads to a rapid restoration of EPC levels.	51,52

CKD, chronic kidney disease; EPC, endothelial progenitor cell; HDL, high-density lipoprotein; LVH, left ventricular hypertrophy; oxLDL, oxidized low-density lipoprotein.

4. CONCLUSIONS

A crucial target in the treatment or prevention of atherosclerosis is to promote and maintain the integrity and health of endothelium. Since EPCs play a role in maintaining an intact and functional endothelium, decreased and dysfunctional EPCs may contribute to endothelial dysfunction and susceptibility of atherosclerosis. The results of these studies indicate that EPCs are important for vascular health, thus advocating research into the underlying mechanisms that are responsible for impaired EPC count and function in various vascular diseases. A clear understanding of EPC biology is of particular relevance to cardiovascular diseases, as it may provide additional insight into the pathogenesis of these diseases, as well as novel targets for therapeutic agents.

ACKNOWLEDGEMENTS

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