Decreased Number and Impaired Angiogenic Function of Endothelial Progenitor Cells in Patients With Chronic Renal Failure

Jin-Ho Choi, Koung Li Kim, Wooseong Huh, Beom Kim, Jonghoe Byun, Wonhee Suh, Jidong Sung, Eun-Seok Jeon, Ha-Young Oh, Duk-Kyung Kim

Objective—Increased risk of cardiovascular disease in patients with chronic renal failure (CRF) has been explained by accelerated atherosclerosis and impaired angiogenesis, in which endothelial progenitor cells (EPCs) may play key roles. We hypothesized that altered EPC biology may contribute to the pathophysiology of CRF.

Methods and Results—EPCs were isolated from CRF patients on maintenance hemodialysis (n=44) and from a normal control group (n=30). CRF patients showed markedly decreased numbers of EPC (44.6%) and colonies (75.3%) when compared with the controls (P<0.001). These findings were corroborated by 30.5% decrease in EPC migratory function in response to vascular endothelial growth factor (VEGF) (P=0.040) and 48.8% decrease in EPC incorporation into human umbilical vein endothelial cells (HUVEC) (P<0.001). In addition, Framingham’s risk factor score of both CRF (r=−0.461, P=0.010) and normal group (r=−0.367, P=0.016) significantly correlated with the numbers of EPC. Indeed, the number of circulating EPC was significantly lower in CRF patients than in normal group under the same burden of risk factors (P<0.001). A significant correlation was also observed between dialysis dose (Kt/V) and EPC incorporation into HUVEC (r=−0.427, P=0.004).

Conclusions—EPC biology, which is critical for neovascularization and the maintenance of vascular function, is altered in CRF. Our data strongly suggest that dysfunction of circulating EPC has a role in the progression of cardiovascular disease in patients with CRF. (Arterioscler Thromb Vasc Biol. 2004;24:1246-1252.)

Key Words: endothelial progenitor cell ■ risk factors ■ vascular biology ■ renal physiology
might be numerically or functionally impaired in CRF. This would contribute to the accelerated atherosclerosis and impairment of angiogenesis, which may account for the increased cardiovascular risk and poor clinical outcomes observed in CRF. We investigated the number and angiogenic function of EPC obtained from patients on maintenance hemodialysis and compared them with the corresponding parameters of healthy volunteers.

**Methods**

**Study Subjects**

We studied 44 male CRF patients on maintenance hemodialysis and 30 healthy male volunteers who were older than age 21 years. Chronic stable patients on maintenance hemodialysis were selected to exclude potential factors that may affect biology of circulating EPC in the setting of recent aggravation of renal function. Patients with clinical evidence of symptomatic coronary artery disease, peripheral vascular disease, cerebrovascular disease, or carotid artery stenosis were excluded. Subjects with any condition, such as neoplasms, wounds, or significant retinopathy, which might involve neovascularization, were also excluded. Medications, including statins, glucose-controlling drugs, and antihypertensive drugs, were continued. No new medications, including vitamins, were used for at least 2 weeks before the study. Laboratory data of each subject were obtained within 2 weeks from the date of blood sampling. All enrolled subjects underwent a detailed assessment of cardiovascular risk after signing an informed consent document approved by the Institutional Review Board of Samsung Medical Center.

**Isolation of EPC**

Peripheral blood (30 mL) was drawn by venipuncture using a heparin-coated syringe. Samples of CRF patients were drawn just before the beginning of hemodialysis to exclude any possible influence of dialyzer on the cell. Mononuclear cells were isolated by density gradient method using Ficoll-Paque Plus (Amersham, Buckinghamshire, UK), and resuspended in EGM-2 MV Singlequot medium (Cambrex, East Rutherford, NJ), which contains multiple growth factors including human vascular endothelial growth factor (VEGF) A, human fibroblast growth factor-2, human endothelial growth factor, insulin-like growth factor-1, and ascorbic acid. Cells were plated on 6-well plates (Becton Dickinson, Franklin Lakes, NJ) coated with 2% gelatin (Sigma, St. Louis, Mo). The initial seeding density was standardized at $4 \times 10^5$ cells per well. After 3 days of culture, nonadherent cells were removed and the media was changed. The culture was maintained through day 7. After the numbers of EPC and EPC colony were counted, cells were assayed or harvested for further study.

**EPC Characterization**

Endothelial cell (EC) phenotype of EPC was investigated at day 7. Adherent cells were incubated with 1:1–diodotyrosine-3,3',3''-tetra-methylindocarbocyanine perchlorate (DiI)-labeled acetylated LDL (Molecular Probes, Eugene, Ore) at 37°C for 3 hours, and then with fluorescein-isothiocyanate-conjugated Ulex europaeus agglutinin (UEA)-1 lectin (10 μg/mL, Sigma) for 4 hours. Samples were examined with an inverted fluorescence microscope (Zeiss) and only cells exhibiting double fluorescence were identified as EPC. Cultured human umbilical vein endothelial cells (HUVEC) and NIH 3T3 cells were stained simultaneously and served as the positive and negative controls, respectively. To confirm endothelial cell phenotype of EPC, cells were also simultaneously stained with primary antibodies against the VEGF receptor KDR (Sigma) and von Willebrand factor (vWF) (BD Pharmingen, San Diego, Calif). Fluorescein-isothiocyanate and rhodamine-linked secondary monoclonal antibodies were used.

To confirm that the EPCs of our study population were capable of intracellular nitric oxide (NO) synthesis, we examined the EPCs of normal subjects with a membrane NO-specific fluorescence indicator, diamino-fluorescein-2 diacetate (DAF-2 DA; Daiichi, Japan). EPCs were gently washed twice with calcium-free phosphate-buffered saline (PBS) and bathed in Krebs-Henseleit buffer containing L-arginine (1 mmol/L) and DAF-2 DA (10 μmol/L) for 15 minutes. The cells were then incubated at 37°C for an additional 15 minutes, and 50 ng/mL of recombinant human VEGF$_{165}$ (R&D Systems, Minneapolis, Minn) was added to the wells. The samples were then examined with fluorescence microscopy.

Cell surface antigens were investigated with fluorescence-activated cell sorter (FACS) analysis. Cultured cells were detached by brief incubation with trypsin/1 mmol/L EDTA followed by forceful pipetting, and cells ($1 \times 10^6$ cells each) were used for FACS analysis. Antibodies to the VEGF receptor KDR (Sigma), VWF (BD Pharmingen), and VE-cadherin (BD Pharmingen) were used as primary antibodies. Peripheral blood mononuclear cells served as controls.

**EPC Count**

The numbers of EPC and EPC colony were determined by counting 12 random high-power ($\times 100$) microscope fields per subject and were expressed as cells or colonies per mm$^2$. Spindle-shaped cells and colonies consisting of multiple thin, flat cells emanating from a central cluster of rounded cells were counted.

**EPC Migration Assay**

EPC migratory function, which is essential for angiogenesis, was examined using a modified Boyden chamber technique. A 24-well Transwell apparatus (Coster) was used, with each well containing a 6.5-mm polycarbonate membrane with 8-μm pores, coated with type I collagen (Sigma). EPCs ($4 \times 10^5$) were placed on the membrane, and the chamber was immersed in a 24-well plate, which was filled with growth factor-free EBM-2 culture media or EBM-2 with 50 ng/mL of human VEGF$_{165}$. After incubation for 24 hours, the membrane was washed briefly with PBS and the upper side of membrane was wiped gently with a cotton ball. The membrane was then removed and stained using Giemsa solution. The magnitude of EPC migration was evaluated by counting the migrated cells in 4 random high-power ($\times 100$) microscope fields.

**Matrigel Tube Formation Assay**

A Matrigel tube formation assay was performed to assess the ability of EPC to incorporate into endothelial cell vascular structures, which is believed to be important in new vessel formation. Matrigel (Becton Dickinson) was spread onto 4-well chamber slides (Nalge Nunc). EPCs were marked with DiI (Molecular Probes) to distinguish them from HUVEC. DiI-labeled EPC ($4 \times 10^5$) and HUVEC ($4 \times 10^5$) were plated together and incubated at 37°C for 24 hours with EGM-2 culture medium. Incorporated cells were counted from 4 random high-power ($\times 100$) microscope fields per each subject. The numbers and line lengths of the circle formed by cells were also calculated.

**Statistical Analysis**

All data are presented as means±SEM. Continuous variables were evaluated by nonparametric Mann–Whitney test or Wilcoxon signed rank test. Categorical variables were evaluated by Fischer exact test. Univariate correlations were made with Spearman correlation coefficient. Multiple linear regression for the analysis of covariance was used to identify predictors of changes in EPC counts in a multivariate setting. SPSS release 11.0 (SPSS Inc) was used, and differences were considered statistically significant at 2-tailed P<0.05.

**Results**

**Patients Characteristics**

Clinical data of the study subjects are summarized in the Table. Patients in the CRF group were 4.6 years older than those in the normal group and had a lower body mass index. Total cholesterol and high-density lipoprotein cholesterol
were significantly lower in the CRF group. All CRF patients were treated with erythropoietin, and most of them (97.7%, n=43) were using antihypertensive medication. Plasma VEGF levels measured with an enzyme-linked immunosorbent assay kit (R&D Systems) showed no difference between CRF and normal groups. There was no difference in total leukocyte counts between 2 groups. The underlying cause of CRF was diabetic nephropathy (n=22) or other causes (n=22).

EPC Characterization
Culture of peripheral blood mononuclear cell resulted in the emergence of characteristic spindle-shaped EPCs (A) and EPC colonies characterized by a central cluster of rounded cells surrounded by radiating spindle-shaped cells (B). EPCs (C through E) and EPC colonies (F to H) were shown to uptake acetylated LDL (C, F) and bind UEA-1 (D, G). Merged images show that most cells are dual-positive (E, H).

EPC Count
Significantly fewer EPC colonies were formed from mononuclear cell culture of CRF patients compared with normal controls at day 7 (133.7±21.6 versus 542.5±64.1 per 10^3 mm²; P<0.001) (Figure 2A through 2C). Significantly fewer EPCs were also identified from CRF patients (10.0±1.2×10^3 versus 17.8±2.0×10^3 per 10^3 mm²; P<0.001) (Figure 2D through 2F). Despite differences in age, body mass index, total cholesterol, high-density lipoprotein cholesterol, hypertension, and usage of statin, which might

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**Table 1. Characteristics of Study Subjects**

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<th>Normal</th>
<th>CRF (DM)</th>
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<td>Age (year)</td>
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<td>BMI (kg/m²)</td>
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<td>Total cholesterol (mg/dl)</td>
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<td>134.1±4.3</td>
<td>125.4±4.9</td>
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<td>HDL-cholesterol (mg/dl)</td>
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<td>Hypertension (%)</td>
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<td>97.7 (43)</td>
<td>95.5 (21)</td>
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<td>Diabetes (%)</td>
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<td>52.3 (23)</td>
<td>100 (22)</td>
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<td>Smoker (%)</td>
<td>36.7 (11)</td>
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<td>(4.5 (1))</td>
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<td>Dialysis duration (year)</td>
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<td>3.6±0.6 (22)</td>
<td>5.0±0.9 (22)</td>
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<td>Use of statin (%)</td>
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<td>(36.4 (8))</td>
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<td>Use of erythropoietin (%)</td>
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<td>100 (44)</td>
<td>(100 (22))</td>
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<td>Plasma VEGF (pg/ml)</td>
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<td>32.6±3.2</td>
<td>(38.6±4.8)</td>
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<td>WBC (/mm³)</td>
<td>6434±228</td>
<td>6142±329</td>
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Data are mean±SEM. Mann–Whitney test for continuous variables and Fischer’s exact test for categorical variables. P-values are derived from the comparison of the normal and CRF group. CRF indicates chronic renal failure; DM, diabetes mellitus; BMI, body mass index; HDL, high density lipoprotein; VEGF, vascular endothelial growth factor; WBC, peripheral blood white blood cell.

*Two-tailed P-value<0.05.
affect EPC biology (Table), the statistically significant differences were maintained after adjusting for these factors (EPC colonies of CRF versus normal; \( P < 0.001 \), EPC of CRF versus normal; \( P < 0.005 \)) (Figure IIA and IIB, available online at http://atvb.ahajournals.org). There was no difference in the numbers of EPCs or EPC colonies between diabetic and nondiabetic CRF patients.

**EPC Migration Assay**

The migratory function of EPC in response to VEGF, which is believed to be important during neovascularization, was evaluated using a modified Boyden chamber. The basal EPC migratory functions of CRF patients and normal controls were not significantly different (\( P > 0.05 \)). After supplementation with 50 ng/mL of VEGF, the migratory function of EPC in both groups was significantly augmented (baseline versus VEGF-augmented migration, CRF patients: 14.9±3.3 versus 22.4±3.7 per \( \times 100 \) high-power field; \( P = 0.001 \), normal group: 19.1±3.2 versus 32.9±5.3 per \( \times 100 \) high-power field; \( P = 0.016 \)) (Figure 3A through 3E). However, the VEGF-induced augmentation of EPC migration was relatively impaired in CRF. VEGF-induced augmentation of EPC migration was reduced by 52.8% in CRF patients relative to that of the normal group (7.5±1.4 versus 15.8±3.5 per \( \times 100 \) high-power field, \( P = 0.036 \)) (Figure 3F), and the total number of migrated EPC was also lower in CRF patients independently of clinical factors described (22.4±3.7 versus 32.9±5.3 per \( \times 100 \) high-power field, \( P = 0.017 \)) (Figure IIC, available online at http://atvb.ahajournals.org).

**Matrigel Tube Formation Assay**

EPC incorporation into the tubular networks formed by HUVEC was evaluated in culture using Matrigel, which is used to evaluate EC differentiation. Significantly fewer EPCs of CRF patients were incorporated into tubules compared with the EPCs from normal controls (8.2±0.5 per \( \times 10^{-3} \) per \( \text{mm}^2 \) versus 16.0±1.1 per \( \times 10^{-3} \), \( P < 0.001 \)) (Figure 4A through 4C), independently of clinical factors described (\( P < 0.001 \)) (Figure IID, available online at http://atvb.ahajournals.org). Significantly fewer circles and shorter line lengths of the tubules were also identified (Figure IIIA through IIDD, available...
online at http://atvb.ahajournals.org). There was no difference in EPC incorporation or tubule formation between diabetic and nondiabetic CRF.

Risk of Coronary Artery Disease and the Level of EPC

We next investigated the relationship between the level of circulating EPC and the risk of coronary artery disease, which is a clinical consequence of accelerated atherosclerosis and impaired angiogenesis in CRF.\(^2,3,4,5,8\) Because none of our study subjects showed any clinical evidence of coronary artery disease, the 10-year coronary artery disease risk was estimated from the total burden of risk factors according to the Framingham risk score.\(^2,20,26\) The EPC number was significantly reduced from the total burden of risk factors according to the disease, the 10-year coronary artery disease risk was estimated from the Framingham risk score (A) and those of the normal controls (B). EPC incorporation in the CRF group was significantly reduced by 51.9% (C). \(^*P<0.05\).

Figure 4. EPC incorporation into EC and stimulation of EC tube formation is impaired in CRF. EPCs were labeled with Dil and cocultured with HUVEC in Matrigel-coated 4-well chamber slide for 24 hours. Fluorescence and light images of identical fields were merged. Significantly fewer EPCs from CRF patients were incorporated into the tubules formed by HUVEC (A) than those of the normal controls (B). EPC incorporation in the CRF group was significantly reduced by 51.9% (C). \(^*P<0.05\).

To compare the EPC numbers in both groups under the same risk burden, the age-adjusted relative risk of each subject was estimated.\(^26\) EPC numbers were also significantly inversely correlated with the age-adjusted relative risk, both in CRF patients \((r=-0.422, P=0.005)\) and in the normal group \((r=-0.399, P=0.029)\). The numbers of EPC were significantly lower in CRF patients after adjustment for risk burden. \((r=-0.367, P=0.016)\) in the total CRF group but also in the low-risk \((r=-0.461, P=0.010)\) and high-risk subgroup \((r=-0.427, P=0.004)\) (Figure 5C). From these observations, it would appear that the level of EPC is reduced in CRF irrespective of the risk burden.

Figure 5. The level of circulating EPC is inversely correlated with the risk factor score of coronary artery disease, and the EPC incorporation function is correlated with the dose of dialysis (Kt/V). The correlation between the number of EPC and the 10-year coronary artery disease risk estimated from the Framingham risk score was evident in the normal group (A) and CRF group (B). C. The age-adjusted relative risk of coronary artery disease was calculated to compare the numbers of EPC under the same risk burden. The correlation was maintained in the normal \((r=-0.439, P=0.029)\) and CRF group \((r=-0.422, P=0.009)\), and there were significantly fewer EPCs in the CRF group than in the normal group after correction for risk burden \((P<0.001)\). D. The number of EPC was significantly reduced not only in the total CRF group but also in the low-risk \((P=0.009)\) and high-risk subgroup \((P<0.001)\). The EPC incorporation function was significantly different between lower doses and higher doses of dialysis \((P=0.015)\) (E) and correlated significantly with the dose of dialysis \((r=0.427, P=0.004)\) (F).

Impact of Dialysis Dose on the EPC Migratory Function

As shown, a lower number of EPC was correlated with a higher future coronary artery disease risk. We presumed that the number or function of EPC might also be related to the dose of dialysis calculated by Kt/V, which is known to be related to mortality risk in CRF patients.\(^27\) Because a Kt/V of 1.3 is regarded as the minimal standard for adequate dialysis,\(^2,27\) we compared the EPC incorporation function of patients receiving a lower dialysis dose (Kt/V < 1.3) with that of patients receiving a higher dialysis dose (Kt/V ≥ 1.3). The EPCs of patients receiving lower doses of dialysis showed significantly impaired incorporation into EC compared with those of patients receiving higher doses of dialysis.
(5.3±1.1×10³ versus 8.6±0.5×10¹ EPC per 10³ mm², 
\(P=0.015\)) (Figure 5E). Furthermore, the dose of dialysis 
was significantly correlated with the degree of incorporation 
of EPC into EC \((r=0.427, P=0.004\) ) (Figure 5F). This 
interesting finding suggests that higher doses of dialysis may 
be related to improved angiogenic function of EPC. However, 
other parameters such as the number of EPC, the number 
of EPC colonies, and migratory functions were not correlated 
with the dose of dialysis.

Discussion
In this study, we demonstrate that the number of circulating 
EPC is decreased and the angiogenic activity of EPC is 
impaired in CRF, a disease in which endothelial dysfunction 
and impaired angiogenesis have been described.\(^8\)\(^{,}\)\(^{,}\)\(^{,}\)\(^{28}\) \(T\) here is 
growing evidence that bone marrow-derived EPC participates 
in the repair of endothelial dysfunction.\(^15\)\(^{,}\)\(^{,}\)\(^{,}\)\(^{,}\)\(^{16}\)\(^{,}\)\(^{,}\)\(^{20}\) This process 

can be divided into 3 stages: mobilization from bone marrow, 

homing into the sites of vascular injury, and incorporation 
into the endothelium of the injured or newly formed blood 
vessels.\(^29\) \In our study, the number of EPC is decreased in 
CRF despite no difference in plasma VEGF levels. This 
implies that there may be a fundamental impairment in the 
production, mobilization, or half-life of EPC in CRF. The 
exhaustion of a presumed finite supply of EPC by continuous 
endothelial damage and consumption of EPC for vascular 
endothelial repair might also contribute to the decreased EPC 
numbers.\(^18\)\(^{–}\)\(^{20}\)

Current data suggest that EPC may be incorporated into 
damaged endothelium and may work in concert with existing 
endothelial cells to form blood vessels rather than forming 
entirely new vessels.\(^18\)\(^{–}\)\(^{20}\)\(^{,}\)\(^{30}\) \It is interesting that not only EPC 
migration and incorporation into EC was reduced but that EC 
network formation was also reduced when EC was cocultured 
with the EPC of CRF patients. These observations suggest 
that EPC incorporation into damaged endothelium or neovas-

cularization foci may be impaired in CRF, and that the 
differentiation of normal EC may also be affected when EPC 
is functionally impaired. These phenomena could contribute 
to the deterioration in the repair of damaged endothelium or 
angiogenesis in CRF.\(^8\)\(^{,}\)\(^{,}\)\(^{28}\)

In our study, the number of EPC was much lower in the 
CRF group than in the normal group, even when both groups 
had the same burden of risk factors. Furthermore, the number 
of EPC was inversely correlated with the estimated future 
coronary artery disease risk in both groups. These findings 
are consistent with the clinical observation that coronary 
artery disease risk in CRF is disproportionately high relative 
to the underlying risk factor profile.\(^9\)\(^{,}\)\(^{10}\) \Our study suggests 
that EPC mobilization and incorporation into damaged endo-
thelium or neovascularization foci may be impaired in CRF, 
as it is in other traditional risk factors such as aging, diabetes, 
and hypercholesterolemia.\(^18\)\(^{–}\)\(^{22}\)\(^{,}\)\(^{31}\)

The dose of dialysis is an index of the removal of uremic 
toxins and is a key determinant of prognosis for end-stage 
CRF patients.\(^1\)\(^{,}27\)\(^{,}32\)\(^{,}33\) \Our data show that the dose of dialysis is 

inversely related to the capacity for EPC incorporation, 

which is a principal step in tissue vascularization by bone 
marrow-derived progenitor cells.\(^16\) \This result may indicate 
the mechanism by which the greater removal of uremic toxins 
by higher doses of dialysis can improve the angiogenic 
function of EPC and the prognosis of CRF patients.

Possible mechanisms for the altered biology of EPC 
observed in CRF are presented here. Excessive oxidative 
stress, which is known to be related to increased cardiovas-
cular risk in CRF, may inhibit the differentiation of EPC into 
the mature EC and contribute to the impaired repair of injured 
vascular endothelium.\(^34\)\(^{,}35\) Another candidate is the deficient 
NO production in CRF.\(^36\)\(^{,}37\) 

NO synthase, which has an 
esential role in mobilization of EPC,\(^28\) is blocked by endog-

enous inhibitory actions of guanidine compounds, which are 

major components of uremic toxins.\(^39\)\(^{–}\)\(^{40}\) \Therefore, deficient 
NO production might lead to decreased mobilization of EPC 
from bone marrow. Finally, the function of EPC may also be 

impaired by uremic toxins, similar to the way activation of T 
and B lymphocyte is impaired in CRF.\(^41\) \In our study, the 
level of blood urea nitrogen, which represents an approximate 
burden of uremic toxin burden, was weakly correlated to the 
number of EPC \((P=0.06, \text{data not shown})\). However, consid-

\ering the fact that the blood urea nitrogen varies widely 

between measurements and is not an accurate measure of 
uremic toxin burden, the correlation between the level of 
uremic toxin and the number of EPC may be suggestive and 
could hardly conclusive.

Recently a new therapeutic strategy involving the admin-
istration of autologous EPC to increase neovascularization 
has generated great interest.\(^23\)\(^{–}\)\(^{44}\) \The results of the present 
study suggest that if such approach is to reach clinical 
fruition, the function of the transplanted cells must also be 
considered. The effects of therapeutic angiogenesis using 
autologous cell transplantation may not be satisfactory in 
patients with EPC dysfunction, such as diabetic, aged, or 
those with CRF. However, our study shows that higher dose 
of dialysis, which may eliminate the cause of EPC dysfunc-
tion, is related to better EPC function. If the angiogenic 
function of EPC can be improved by modification of the 
pathophysiology of EPC dysfunction, not only the therapeutic 
outcome of EPC transplantation but also the future coronary 
artery disease risk may be improved.

In conclusion, our data demonstrate that EPC is numeri-
cally and functionally impaired in CRF, and that this is 

inversely related to the risk factor score of coronary artery 

disease. The results of our study may account for the 

acceleration of atherosclerosis, impairment of angiogenesis, 

and increased coronary artery disease risk observed in CRF 

patients.

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Figure I.

A  B  C
D  E  F

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<tr>
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The number of EPC colony was different between CRF and normal group, independently of clinical factors.

<table>
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<tr>
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<th>β-coefficient</th>
<th>p-value</th>
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The number of EPC was different between CRF and normal group, independently of clinical factors.

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The number of migrated EPC was different between CRF and normal group, independently of clinical factors.

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The number of incorporated EPC into EC tubules was different between CRF and normal group, independently of clinical factors.
Figure III.

C  circle number

D  line length

Circle per ×100 HPF

Normal  CRF  DM  Non-DM

line length (mm) per ×100 HPF

Normal  CRF  DM  Non-DM
Figure Legends for Supplementary Data

Figure I. Identification of the endothelial lineage phenotype of cultured EPC. A-C, EPCs and EPC colony showed expression of endothelial lineage markers, vWF (A) and KDR (B). Merged image shows simultaneous expression of these markers (C). D-F, EPCs were both positive for vWF (D) and DAF2-DA, a NO synthesis indicator (E). Merged image shows specific NO synthesis in vWF-positive cells (F). G, FACS analysis of EPC cultured for one week showed that EPCs express markers consistent with endothelial lineage (KDR; 23.9%, vWF; 53.3%, VE-cadherin; 39.1%). PB-MNC (peripheral blood mononuclear cell) served as controls. Asterisk (*) denotes statistical significance (p<0.05).

Figure II. The biological difference of EPC between CRF and normal group was sustained independently of clinical factors. The number of EPC colony (p<0.001) (A), the number of EPC (p=0.005) (B), the number of migrated EPC (C), and the number of incorporated EPC into EC tubules (D) were all different between CRF and normal group, and these statistically significant differences were maintained after adjusting for clinical factors. * p<0.05.

Figure III. EPC incorporation into EC and stimulation of EC tube formation is impaired in CRF. Dil-labeled EPCs were co-cultured with HUVEC in Matrigel-coated chamber slide for 24 hours.
Fluorescence and light images of identical fields were merged. A-D, Significantly less tubule formation and shorter tubule line lengths were also identified in the CRF group (A) than in the normal group (B). The graphs show a 27.7% decrease in the number of circles (p=0.001) (C) and a 16.6% decrease in the lengths of circle lines (p=0.029) (D). * p<0.05.