SPECIAL ARTICLE www.jasn.org

Dysfunctional Endothelial Progenitor Cells in Chronic Kidney Disease

Michael S. Goligorsky, Kaoru Yasuda, and Brian Ratliff

Renal Research Institute, Departments of Medicine, Pharmacology and Physiology, New York Medical College, Valhalla, New York

ABSTRACT

Putative endothelial progenitor cells play a role in organ regeneration, and their incompetence may be important in the development of chronic kidney disease. The mechanisms of this incompetence are broad and range from poor mobilization, viability, and engraftment to impaired differentiation into mature endothelial cells. By contrasting the role of endothelial progenitor cells in tissue regeneration with their developing incompetence in chronic kidney disease, we emphasize the importance of designing rational pharmacologic strategies to tackle such incompetence in the broader search for therapies to attenuate chronic disease.

J Am Soc Nephrol 21: 911–919, 2010. doi: 10.1681/ASN.2009111119

A landmark publication identifying circulating endothelial progenitor cells (EPCs) provides new impetus for their use as cell therapy in postnatal vasculogenesis.[1] In this seminal study, human circulating $CD34^+$ cells were instructed to differentiate in culture into cells with endothelial-like properties. Moreover, administration of these cells to athymic nude mice with hindlimb ischemia resulted in the integration of transplanted cells into capillary vessels, improving collateral circulation. The idea that such cells derive from hematopoietic stem cells (HSCs) in bone marrow is also supported in mice, in which transplantation of a single HSC repopulated the bone marrow and the endothelium of retinal blood vessels after experimental retinal ischemia.[2] Comparable results have been published by others.[3]

Embedded in a broad view of EPCs is the notion that angiogenic cells are capable of maturing into endothelial cells (ECs). These cells are mostly angioblastic or myelocytic descendants. Problems with the definition of EPCs reflect the changing landscape of surface markers as they traffic from the bone marrow to the circulation and tissues, gradually losing monocytic and pan-leukocytic markers as they mature into ECs (Table 1). A subset of angiogenic monocytes is characterized as $CD14^+$,$CD16^+$,$Tie2^+$, whereas circulating bone marrow–derived EPCs are $CD34^+$,VEGFR2 (Flk-1)$^+$,$CD45^{low}$. The consensus combination of markers and processes, although individually not unique to EPCs, is that EPCs express CD34, vascular endothelial growth factor receptor 2 (VEGFR2), Tie-2, CD133, and Ulex Europeus lectin binding; take up acetylated LDL; and have the ability to form colonies (colony-forming unit cells). The problems in identifying EPCs and distinguishing them from circulating ECs or mature ECs remain insurmountable and await future recognition of more specific markers; hence, limited definitions constrain all studies describing these cells as putative.

Transplantation of EPCs augments neovascularization of ischemic or infarcted myocardium, ischemic limbs, and brain.[1,4] EPCs play a critical role in maintaining the integrity of vascular endothelia and serve in their repair after injury or inflammation.[5] Comparatively fewer data have been published on EPCs in kidney regeneration. Rookmaaker *et al.*[6] observed a more than four-fold increase in the number of bone marrow–derived glomerular ECs after anti–Thy-1.1 injection into rats that had received a bone marrow transplant. The participation of donor-derived cells in glomerular EC turnover was also observed in rats that had received a bone marrow transplant, undergone unilateral nephrectomy, and received anti–Thy-1.1 injection.[7] In a pig model of renovascular disease, Chade *et al.*[8] demonstrated that intrarenal infusion of autologous EPCs attenuates vascular remodeling and fibrosis, improving renal function by stimulating angiogenesis. In patients with chronic renal insufficiency, numbers of circulating EPCs were almost 30% lower than in healthy control subjects.[9] Collectively, these studies set the stage for in-depth exploration of EPC participation in regenerative processes.

Our knowledge regarding the pool of EPCs in postnatal life is growing. EPCs are present in the bone marrow, where $CD34^+$ cells constitute approximately

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Michael S. Goligorsky, Renal Research Institute, Departments of Medicine, Pharmacology and Physiology, New York Medical College, Valhalla, NY 10595. Phone: 914-594-4730; Fax: 914-594-4732; E-mail: michael_goligorsky@nymc.edu

Table 1. Expression of surface markers

Marker	BMH	HPP	BMEPC	CEPC	CEC	Mature EC
VEGFR2	+	+ or −	+	+	+	+
Scl	+	−	−	−	−	−
CD133	+	?	+	+(early)	−	−
CD34	+ or −	+	+	+	low	−
CD45	+	+	+	+ or −	−	−
CD31	−	−	−	+	+	+
VE-cad	−	−	−	+	+	+
CD14	−	+	+	−	−	−
vWF	−	−	+	+	+	+

BMH, bone marrow hemangioblast; HPP, hematopoietic progenitor; BMEPC, bone marrow EPC; CEPC, circulating EPC; CEC, circulating EC; VE-cad, VE cadherin; vWF, von Willebrand factor.

2% and AC133/CD133$^+$ cells 0.5% of cells. They also exist in the circulation (0.01% of mononuclear cells) and in the vasculogenic zone between the adventitial and medial layers of large and medium-sized blood vessels.[10] This latter niche contributes to the residence of EPCs in many organs, including liver, kidney, heart, and lung. EPCs derived from all of these niches participate in postnatal angiogenesis, vasculogenesis, and tissue regeneration. For instance, liver-derived EPCs (c-kit$^+$CD45$^-$) contribute to neovascularization of ischemic hindlimb.[11] Relations between various EPC pools and their contribution to regenerative and angiogenic processes are depicted in Figure 1. Not only EPCs but also HSCs and mesenchymal stem cells (MSCs) reside in this vascular niche,[12] and it is possible to envisage that transformation from one cell type to another takes place. Refining the local cues guiding these processes is an important task for future studies.

The idea that EPCs become dysfunctional during the course of a disease is predicated on the demonstration of their defective regenerative capacity or compromised ability to form colonies or migrate and form capillary-like structures. Aging is the most common cause of EPC dysfunction.[13] Similar to all somatic cells, EPCs are subject to various environmental and endogenous stressors that impair their competence. Hyperglycemia reduces survival and impairs the function of circulating EPCs.[14] In addition, EPC dysfunction is documented in types 1 and 2 diabetes, coronary artery disease (CAD), atherosclerosis, vasculitis with kidney involvement, and ESRD.[15–20] There is emerging evidence that senescence may serve as an important mechanism mediating EPC dysfunction. Decreasing numbers and increasing proportion of senescent EPCs has been reported in patients with preeclampsia or hypertension,[21,22] and angiotensin II induces EPC senescence through the induction of oxidative stress and its influence on telomerase activity, as does oxidized LDL. Our recent studies showed that bone marrow-derived cells (BMDCs) and bone marrow-derived EPCs from db/db mice with metabolic syndrome and diabetes were functionally incompetent, whereas adoptive transfer of BMDCs from syngeneic nondiabetic controls significantly improved vasculopathy, insulin sensitivity, and renal function in db/db recipients.[23] Specifically, the development of type 2 diabetes and vasculopathy associated with reduced stress tolerance for EPCs, increased frequency of apoptosis, and premature senescence.[23]

There is no clearcut definition of stem cell incompetence. On the basis of the known trafficking patterns of stem cells in general and EPCs in particular, mobilization, recruitment and engraftment, cell-dependent paracrine signaling, asymmetric proliferative capacity, or viability and resistance to existing stressors,[24] one would extrapolate that stem cell incompetence is characterized by at least one of the following features: Know where, know when, and know how. Figure 2 illustrates this cycle of injury to regeneration involving all of these functions of stem cells and sites where these functions can be perturbed.

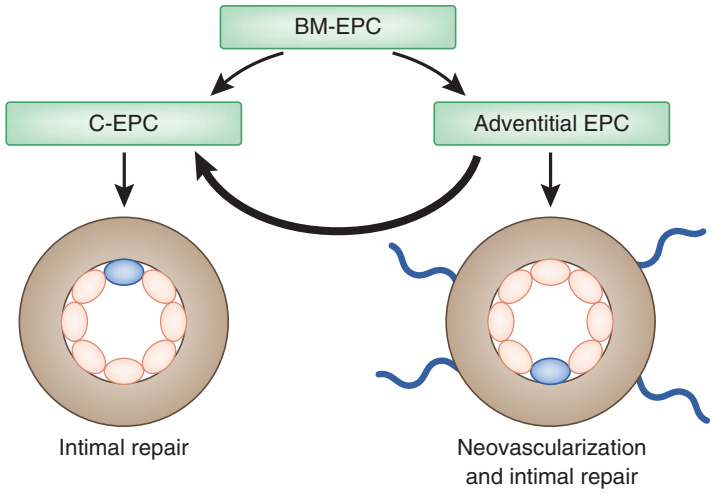

Figure 1. Trafficking and functional potency of EPCs in facilitating intimal repair and induction of neovascularization.

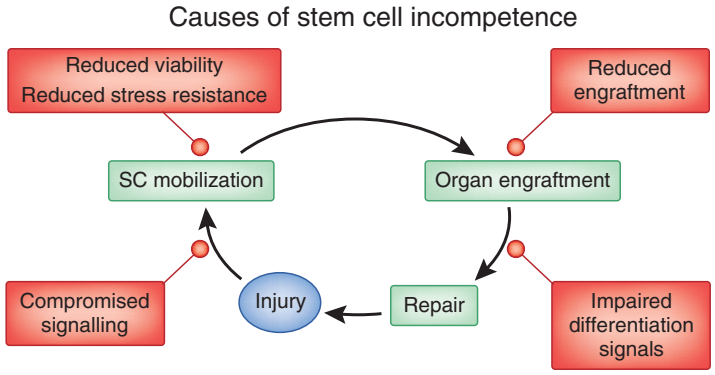

Figure 2. Participation of EPCs in reparative and regenerative processes can be disrupted at different levels: Their mobilization, viability and resistance to stress, engraftment efficiency, and differentiation capacity.

KNOW WHEN TO ENGAGE, OR IMPAIRED MOBILIZATION

Impaired EPC mobilization can occur as a result of inadequacy of mobilizing signals or their shielding or inefficient detachment of stem cells from their niches. A classical mobilization agent, G-CSF, executes dislodgement of stem cells from the bone marrow niche by activating neutrophil elastase, which in turn degrades stromal cell–derived factor 1 (SDF-1).[25] Caveolin 1 deficiency blocks efficient dimerization of CXCR4, whereas caveolin 1 overexpression results in the enhanced SDF-1/CXCR4 binding.[26]

Mobilization of EPCs from their respective niches is accomplished by mechanical injury and ischemic stress through generation of hypoxia-inducible factor 1–regulated release of VEGF, erythropoietin, and SDF-1, as well as by placental growth factor and granulocyte and granulocyte-macrophage colony-stimulating factors.[27–31] Some proinflammatory cytokines, such as IL-8, are potent stimulators of EPC mobilization, thus linking this response to stressors with proinflammatory conditions.[32] An additional mechanism for EPC growth and differentiation has been attributed to apoptotic endothelial microparticles generated after injury to mature ECs,[33] a mechanism that creates a feedback loop to enhance number and initiate the differentiation of EPCs when they are required by the demand for repair.

A number of investigators have examined whether EPCs are efficiently delivered to areas of tissue ischemia to preserve or restore end organ function by participating in vasculogenesis. In a model of myocardial infarction in mice that received a bone marrow transplant, histologic analysis showed donor-derived ECs in areas of neovascularization at the border zone of the infarct.[34] These experimental data are strongly supported by clinical observations. Adams *et al.*[35] found increased levels of circulating EPCs in patients with CAD after exercise-induced myocardial ischemia. Lambiase *et al.*[36] showed an inverse correlation between the density of coronary collaterals and numbers of peripheral EPCs in CAD. Such an inverse correlation between the number and function of circulating EPCs and severity of various chronic cardiovascular and renal diseases seems to be a general rule.[37]

Pharmacologic mobilization of EPCs can be achieved using statins, VEGF, erythropoietin, angiotensin-converting enzyme inhibitors, angiotensin 2 receptor antagonists, peroxisome proliferator–activated receptor γ, G-CSF, and estrogens, to name a few.[38–42] Most of these medications exert their effects, at least in part, through the activation of endothelial nitric oxide synthase (eNOS). Considering this enzyme is usually uncoupled in many diseases, it should come as no surprise that mobilization of EPCs is dependent on functional eNOS. Specifically, mice deficient in the enzyme showed reduced EPC mobilization.[43]

Ischemia is one of the potent signals to mobilize EPCs; this has been unequivocally documented in humans and in experimental animals with myocardial ischemia, ischemic stroke, and renal ischemia.[35,44–47] Despite the seeming universality of this response to ischemic insult, the precise molecular mechanisms responsible for it remain uncertain.

One of the mechanistic uncertainties is related to the question, "What are stress-signaling molecules, produced by and discharged from the ischemic tissue, that are capable of downstream mobilization and recruitment of stem cells and EPCs?" Uric acid, one of the prototypical alarm signals activating the innate immune system, exhibits a short-lived surge after ischemia-reperfusion. Previous studies demonstrated that exogenous uric acid leads to a rapid mobilization of EPCs and HSCs, respectively, and protection of the kidney against ischemic injury.[47] Kuo *et al.*[48] demonstrated that monosodium urate (MSU) *in vitro* and *in vivo* results in exocytosis of Weibel-Palade bodies with the release of IL-8, von Willebrand factor, and angiopoietin 2 into culture medium or the circulation, respectively. In Toll-like receptor 4 null mice, acute elevation of uric acid level by injection of MSU does not result in the release of von Willebrand factor or angiopoietin 2 to the circulation, suggesting that the effect of uric acid on exocytosis of Weibel-Palade bodies is mediated through this receptor. The release of IL-8 in response to elevated uric acid level requires both Toll-like receptors 2 and 4. These findings outline a novel paradigm linking postischemic repair and inflammation by the release of constituents of Weibel-Palade bodies and further broaden the spectrum of alarm signaling that establishes constituents of Weibel-Palade bodies as potential second messengers not only for proinflammatory responses but also for mobilization of stem cells. It has to be emphasized that it is only a brief transient surge of uric acid that has this signaling function. In contrast, chronic elevation of uric acid is completely devoid of this type of alarm signaling; moreover, chronicity inhibits ischemia-induced mobilization.[47]

Interactions between HSCs and stromal cells are mediated in part through $\alpha4\beta1$(VLA4)/VCAM-1.[49] Accordingly, inducible ablation of $\alpha4\beta1$(VLA4) and conditional ablation of VCAM-1 both associate with the enhancement of G-CSF–induced mobilization of HSCs.[50–52] An additional mechanism of leptin-induced upregulation of $\alpha V\beta5$ and $\alpha4$ integrins may explain enhanced adhesion to neointimal lesions,[53] but the same phenomenon seems to inhibit EPC mobilization, thus requiring further analysis. There are multiple remaining questions related to the efficacy of EPC mobilization by intrinsic or pharmacologic means in chronic kidney disease (CKD) that need to be addressed in future studies.

KNOW WHERE TO GO, OR IMPAIRED ENGRAFTMENT

Reduced CXCR4/SDF-1 expression or function impairs engraftment. Homing of HSCs, EPCs, or a subpopulation of MSCs that express CXCR4 is governed mainly by the α-chemokine SDF-1.[54,55] Other potential ligands engaged in homing of stem cells, albeit significantly less explored, include chemokines MCP-3 (homes MSC) and GRO-1 (homes bone marrow–derived EPCs, especially in tumors). Factors affecting binding of SDF-1 to its cognate G-protein–coupled seven-transmembrane domain receptor CXCR4 are many,[54] as summarized in Figure 3, illustrating a plethora of proinflammatory and other factors that disrupt receptor activation. SDF-1/CXCR4 binding activates several pathways, such as focal adhesion kinase (FAK)/Paxillin, phsphatidylinositol-3-kinase/Akt, mitogen-activated or extracellular signal-regulated protein kinase kinase (MEK), Janus kinase/signal transducer and activator of transcription (Jak/STAT), NF-κB, matrix metalloproteinases, and phosphatases Ship 1,2 and CD45, thus regulating cell motility and chemotaxis, adhesion, survival, and secretion. Upon receptor activation, CXCR4 undergoes oligomerization, a process that takes place in lipid-rich domains and requires caveolin 1 expression.[25] Both CXCR4 and SDF-1 are expressed in the normal kidney, and their expression is already enhanced 24 hours after ischemia.[56] Additional and apparently very important factors involved in homing of bone marrow–derived stem cells, EPCs specifically, have been identified as thymosin $\beta4$ and $\alpha4\beta1$ integrin.[57]

Knowledge of where to home is also affected by impaired niche capacities or properties. The idea of stem cell niches is that they provide cell attachment and a microenvironment supporting quiescence by sheltering cells from proliferative and differentiation signals, enhancing cell survival, regulating stem cell division and renewal, and coordinating the population of resident stem cells to meet the actual requirements of an organ.[58] B.R. *et al.* (unpublished observations) addressed the problem of identification of EPC niches by presenting exogenous EPCs to *ex vivo* kidney sections and detecting their adhesion. Kidney ischemia results in more than doubling of the number of adherent cells, suggesting but not proving that the capacity of EPC niche increases under these conditions. Pretreatment of EPCs with TNF-α before their use in adhesion assays reduces their adhesion, whereas hypoxic pretreatment or exposure to elevated concentration of MSU results in a dramatic enhancement of EPC adhesion to ischemic kidney sections (unpublished observations). These differences in adhesion allude to possible changes in the capacity of EPC niches as affected by tissue ischemia or hypoxic preconditioning of EPCs.

KNOW WHAT TO DO, OR IMPAIRED SIGNALING AND MATURATION

Impaired signaling and transformation of EPCs into a mature EC occurs as a result of either perturbed paracrine secretion or differentiation. Ability of circulating progenitors to differentiate toward endothelial or smooth muscle cell lineages has been furthered by studies of parabiosis in which a wild-type mouse and a transgenic mouse expressing green fluorescence protein are conjoined subcutaneously by anastamosing circulations. Tanaka *et al.*[59] demonstrated that mechanical injury to femoral arteries of wild-type mice result within 4 weeks in a chimerism of cells comprising developing neointima: 15 and 31% of parabiotic partner–derived green fluorescence protein–positive cells were detected in the intimal and medial layers, respectively, with some cells expressing α-SMA and others CD31. Diabetes impairs the differentiation potential of bone marrow–derived precursor cells, although the composition of bone marrow does not change, resulting in 40% reduction of

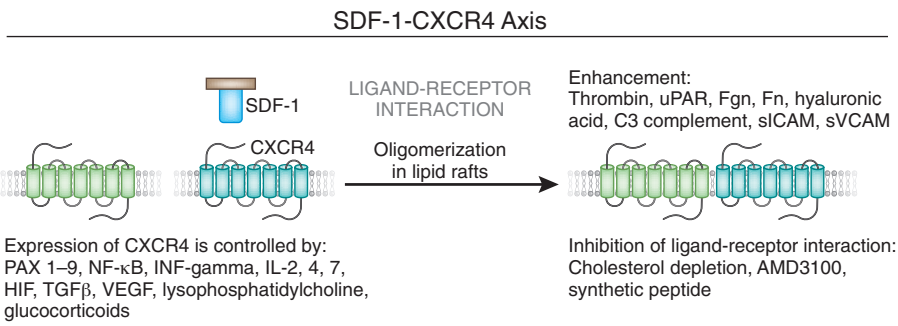

Figure 3. Potential mechanisms modulating SDF-1–CXCR4 interaction and signaling.

EPCs and 50% increase of macrophages, all changes that are correctable by statins.[60] EPC differentiation is inhibited by vascular endothelial growth inhibitor TNFSF15, which decreases the expression of EC markers and induces apoptosis.[61] It is obvious that the differentiation potential of EPCs and their impairment remain poorly investigated.

KNOW HOW TO SURVIVE, OR IMPAIRED SELF-PROTECTION

The loss of EPC viability and resistance to ongoing stress occurs in various disease states. Ito *et al.*[62] convincingly demonstrated that chronic states of oxidative stress induced either by an inhibitor of glutathione synthesis or by deletion of the Atm (ataxia telangiectasia mutated) gene shorten the lifespan of HSCs through increases in proliferation, stem cell exhaustion, and bone marrow failure, expressed as defective bone marrow reconstitution. This is an example of induction of an accelerated type of replicative senescence by reactive oxygen species. Our own data demonstrate that oxidative stress in diabetic db/db mice associates with an increased proportion of apoptotic EPCs under basal conditions and reduced viability under conditions of stress.[23] Similar findings were described for patients with type 2 diabetes: EPCs exhibit impaired proliferation, adhesion, and incorporation into vascular structures.[63] Pharmacologic restoration of stem cell competence, both *ex vivo* and *in vivo* using a selenoorganic antioxidant and peroxynitrite scavenger, ebselen, is possible.[23]

To draw a preliminary conclusion, stem or progenitor cells are differentially affected in different models of CKD. For instance, in adriamycin nephropathy, the prevailing defect traces to the inability of stem or progenitor cell homing to the kidney as a result of the cytotoxic effect of this anthracycline antibiotic; in diabetic nephropathy, the defect is explained predominantly by oxidative stress and reduced viability of stem or progenitor cells; in unilateral ureteral obstruction, pathology of stem or progenitor cell predilection toward fibroblastic differentiation is likely responsible for the ensuing tubulointerstitial fibrosis and scarring. These hypothetical pathogenic mechanisms require in-depth investigation, because the delineation of the causes of stem or progenitor cell incompetence is a prerequisite to designing effective therapies.

THERAPIES TO IMPROVE EPC COMPETENCE

Attempts to improve EPC viability and function are on the way. Genetic engineering for therapy of EPCs *ex vivo* involving overexpression of eNOS or heme-oxygenase 1 resulted in improvement of their function and inhibition of neo-intimal hyperplasia.[64] Overexpression of telomerase reverse-transcriptase in EPCs led to improved neovascularization of ischemic limbs.[65] EPCs stably overexpressing an anti-inflammatory protein, A20, showed resistance to TNF-α and IL-β.[66] Another example of EPC therapy is *ex vivo* or *in vivo* treatment with a peroxynitrite scavenger and glutathione peroxidase mimetic, ebselen, which resulted in a striking improvement in vascular and renal function of db/db mice.[23] Di Stefano *et al.*[67] used a p66ShcA deletion to reduce glucose-induced oxidative stress and restore viability to EPCs. Downregulation of the Ets1 transcription factor in vasculogenic progenitor cells increased their numbers and differentiation to ECs in a mouse model of metabolic syndrome and type 2 diabetes, the lepr(db) mouse.[68] Improved differentiation of EPCs into ECs was achieved by overexpression of a constitutively active form of protein kinase A, which led to induction of Flk-1 and neuropilin 1.[69] These as yet tentative mechanistic attempts at correcting EPC incompetence and improving their survival await future refinement and verification.

POTENTIAL MECHANISMS OF EPC-INDUCED ORGAN REPAIR

EPC participation in reparative processes has been the subject of several excellent reviews[70–72] and therefore is discussed here only briefly.

EPC Incorporation

Direct incorporation of EPCs into a presumably defective intimal layer was demonstrated by Asahara *et al.*[1] EPC transplantation into diabetic mice resulted in vascular engraftment and restoration of blood flow in hindlimb ischemia.[73] Cross-grafting aortic segments between Balb/c and Tie-2/LacZ mice demonstrated chimerism of ECs in the intimal layer, thus arguing in favor of EPC incorporation into vessels.[52] This process is facilitated in part by platelet adhesion at the site of vascular injury, resulting in adhesion and maturation of circulating EPCs to ECs.[74] Usually, direct EPC engraftment varies within 1 to 25%.[75] There is growing concern whether BMDCs or EPCs actually repair endothelia,[76,77] with the emphasis shifting toward progenitors within the vascular wall.

Paracrine Effects of EPCs

Cultured peripheral cells or BMDCs give rise to at least two populations of EPCs: Early VEGFR2$^+$ and VE-cadherin$^+$ cells coexpressing myeloid CD14 and pan-leukocytic CD45 markers after 4 to 7 days and late outgrowth cells emerging after 2 to 3 weeks from CD14$^-$ nonmyeloid populations expressing CD34, VEGFR2, and AC133.[70–72] Both populations are capable of inducing neovascularization, but the mode of action differs. Whereas early outgrowth EPCs have limited capacity for population doubling and induce transient angiogenesis, late outgrowth EPCs expand to more than 100 population doublings. Cell therapy with both populations resulted in the enhanced engraftment and neovascularization during hindlimb ischemia.[70–72] Early outgrowth EPCs exert angiogenic effects mainly by secretory products, whereas late outgrowth cells do this by direct engraftment. The secretome of EPCs using a combination of proteomic techniques reveal angiogenic factors such as thymidine phosphorylase as a major agonist, matrix metalloproteinase 9, IL-8, pre–B cell enhancing factor, and macrophage migration inhibitory factor, among others.[78]

Cell–Cell Fusion

Fusion events have been elegantly documented between BMDCs and tubular epithelial cells after ischemic injury.[79] Notably, this mechanism of repair occurs on a one-to-one basis. The ability of EPCs to fuse with renal resident cells remains to be established.

Tunneling Nanotubes

The formation of tunneling nanotubles between cultured cells is a viable mechanism of organellar exchange between the partners (Figure 4).[80] This mechanism accounts for mitochondrial transfer between adult stem cells and somatic cells to rescue respiration.[81] This mechanism, although difficult to demonstrate *in vivo* and therefore studied only in cultured cells, plays a significant role in intercellular communication.[82] K.Y. *et al.* (unpublished observations) co-cultured human umbilical vein ECs (HUVECs) with EPCs, each cell type labeled with differentially emitting fluorophores, and observed that exchange occurred under basal conditions. EPC-to-HUVEC flux, however, increased threefold after exposure of HUVECs to a cytotoxic dose of adriamycin (unpublished data). Tunneling nanotuble mechanisms of organellar exchange may provide the means for the single EPCs to exchange organelles with multiple HUVECs. Disease processes, resulting in aberrant regeneration even when other functions of EPCs remain intact, may target each of these mechanisms.

USE OF ARTIFICIAL NICHES TO STORE AND DELIVER EPCs

One of the stumbling blocks in stem cell therapy is the problem of low percentages of transplanted cells that survive in the circulation or after tissue engraftment. Delivering cell therapy in synthetic scaffolds may circumvent this problem. The cornerstone idea behind artificial stem cell niches investigated thus far is in the generation of scaffolds with properties of extracellular matrix.[83] Creation of artificial stem cell niches represents an attempt to mimic the natural niche by providing cells with a low-oxygen environment within these avascular scaffolds but ensuring their ability to preserve phenotype, quiescence, recruitability, and protection from noxious stimuli. Hyaluronic acid hydrogels are capable of storing, propagating, and differentiating stem cells.[84] We have initiated studies of EPCs encapsulated in an artificial stem cell niche manufactured from polymeric hyaluronic acid and demonstrates that it is a dynamic storage compartment that not only preserves cells and improves resistance of EPCs to cytotoxic and genotoxic insults but also allows the recruitment of stem or progenitor cells on demand and improves their engraftment to affected organs and preserves their regenerative potential.

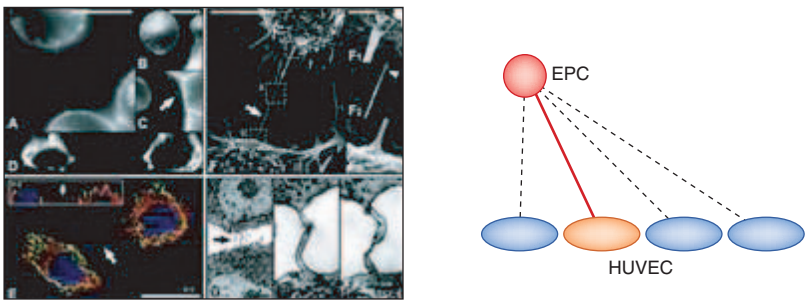

Figure 4. Architecture of tunneling nanotubles (TNTs) between cultured PC12 cells. Wheat germ agglutinin–stained PC12 cells are analyzed by three-dimensional (3-D) live-cell microscopy. (A and B) Cells are connected *via* one (A) or several (B) TNTs with surrounding cells. (C) Rarely, branched TNTs are observed (arrow). (D) A selected (*x-z*) section obtained from a confocal 3-D reconstruction. (E) TNTs contain actin but no microtubules. Fixed PC12 cells were immunostained with an antibody against α-tubulin (green), phalloidin-FITC (red), and DAPI (blue). A single (*x-y*) section of a deconvolved 3-D reconstruction. The inset depicts the corresponding (*x-z*) section through the marked TNT (arrow). (F and G) Ultrastructure of TNTs. PC12 cells analyzed by scanning electron microscopy (F) or transmission electron microscopy (G) of consecutive 80-nm sections. Reprinted from Rustom *et al.*[80], with permission. (Right) A schematic illustration of a possible EPC repair of damaged HUVECs by a repeated formation of TNT between a single EPC and several HUVECs.

CONCLUSIONS

Two lines of evidence collide in this brief essay: On the one hand, the growing evidence of participation of EPCs in regenerative processes and, on the other, the development of EPC incompetence in various disease states. The prognostic value of circulating EPCs has been used to monitor endothelial dysfunction in critically ill patients.[85] Evidence of EPC dysfunction in CKD continues to accrue.[86,87] It is an urgent task for future studies to characterize the mechanisms of EPC dysfunction and design rational therapeutic strategies to restore their competence.

ACKNOWLEDGMENTS

These studies were supported in part by National Institutes of Health grants DK54602, DK052783, and DK45462 (M.S.G.) and Westchester Artificial Kidney Foundation.

DISCLOSURES

None.

REFERENCES

1. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM: Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275: 964–967, 1997
2. Grant MB, May WS, Caballero S, Brown GA, Guthrie SM, Mames RN, Byrne BJ, Vaught T, Spoerri PE, Peck AB, Scott EW: Adult hematopoietic stem cells provide functional hemangioblast activity during retinal neovascularization. *Nat Med* 8: 607–612, 2002
3. Bailey AS, Jiang S, Afentoulis M, Baumann CI, Schroeder DA, Olson SB, Wong MH, Fleming

WH: Transplanted adult hematopoietic stems cells differentiate into functional endothelial cells. *Blood* 103: 13–19, 2004
4. Zhang ZG, Zhang L, Jiang Q, Chopp M: Bone marrow-derived endothelial progenitor cells participate in cerebral neovascularization after focal cerebral ischemia in the adult mouse. *Circ Res* 90: 284–288, 2002
5. Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I: Endothelial cell senescence in human atherosclerosis: Role of telomere in endothelial dysfunction. *Circulation* 105: 1541–1544, 2002
6. Rookmaaker MB, Verhaar MC, van Zonneveld AJ, Rabelink TJ: Progenitor cells in the kidney: Biology and therapeutic perspectives. *Kidney Int* 66: 518–522, 2004
7. Ikarashi K, Li B, Suwa M, Kawamura K, Morioka T, Yao J, Khan F, Uchiyama M, Oite T: Bone marrow cells contribute to regeneration of damaged glomerular endothelial cells. *Kidney Int* 67: 1925–1933, 2005
8. Chade A, Zhu X, Lavi R, Krier J, Pislaru S, Simari R, Napoli C, Lerman A, Lerman L: Endothelial progenitor cells restore renal function in chronic experimental renovascular disease. *Circulation* 119: 547–557, 2009
9. de Groot K, Bahlmann FH, Sowa J, Koenig J, Menne J, Haller H, Fliser D: Uremia causes endothelial progenitor cell deficiency. *Kidney Int* 66: 641–646, 2004
10. Ergun S, Tilki D, Hohn H, Gehling U, Kilic N: Potential implications of vascular wall resident endothelial progenitor cells. *Thromb Haemost* 98: 930–939, 2007
11. Aicher A, Rentsch M, Sasaki K, Ellwart JW, Fändrich F, Siebert R, Cooke JP, Dimmeler S, Heeschen C: Non-bone marrow-derived circulating progenitor cells contribute to postnatal vascularization following tissue ischemia. *Circ Res* 100: 581–589, 2007
12. Kiel M, Yilmaz O, Iwashita T, Yilmaz O, Terhorst C, Morrison S: SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* 121: 1109–1121, 2005
13. Rauscher FM, Goldschmidt-Clermont PJ, Davis BH, Wang T, Gregg D, Ramaswami P, Pippen AM, Annex BH, Dong C, Taylor DA: Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation* 108: 457–463, 2003
14. Krankel N, Adams V, Linke A, Gielen S, Erbs S, Lenk K, Schuler G, Hambrecht R: Hyperglycemia reduces survival and impairs function of circulating blood-derived progenitor cells. *Arterioscler Thromb Vasc Biol* 25: 698–703, 2005
15. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, Zeiher AM, Dimmeler S: Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 89: E1–E7, 2001
16. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Bohm M, Nickenig G: Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* 353: 999–1007, 2005
17. Loomans CJ, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, Verhaar MC, Braam B, Rabelink TJ, van Zonneveld AJ: Endothelial progenitor cell dysfunction: A novel concept in the pathogenesis of vascular complications of type 1 diabetes. *Diabetes* 53: 195–199, 2004
18. Holmen C, Elsheikh E, Stenvinkel P, Qureshi AR, Pettersson E, Jalkanen S, Sumitran-Holgersson S: Circulating inflammatory endothelial cells contribute to endothelial progenitor cell dysfunction in patients with vasculitis and kidney involvement. *J Am Soc Nephrol* 16: 3110–3120, 2005
19. Chan CT, Li SH, Verma S: Nocturnal hemodialysis is associated with restoration of impaired endothelial progenitor cell biology in end-stage renal disease. *Am J Physiol Renal Physiol* 289: F679–F684, 2005
20. Choi JH, Kim KL, Huh W, Kim B, Byun J, Suh W, Sung J, Jeon ES, Oh HY, Kim DK: Decreased number and impaired angiogenic function of endothelial progenitor cells in patients with chronic renal failure. *Arterioscler Thromb Vasc Biol* 24: 1246–1252, 2004
21. Sugawara J, Mitsui-Saito M, Hayashi C, Hoshiai T, Senoo M, Chisaka H, Yaegashi N, Okamura K: Decrease and senescence of endothelial progenitor cells in patients with preeclampsia. *J Clin Endocrinol Metab* 90: 5329–5332, 2005
22. Imanishi T, Moriwaki C, Hano T, Nishio I: Endothelial progenitor cell senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension. *J Hypertens* 23: 1831–1837, 2005
23. Chen J, Li H, Addabbo F, Zhang F, Pelger E, Patschan D, Kuo MC, Gobe G, Nasjletti A, Goligorsky MS: Adoptive transfer of syngeneic bone marrow-derived cells in mice with obesity-induced diabetes: Selenoorganic antioxidant ebselen restores stem cell competence. *Am J Pathol* 174: 701–711, 2009
24. Dimmeler S, Zeiher A: Vascular repair by circulating endothelial progenitor cells: The missing link in atherosclerosis? *J Mol Med* 82: 671–677, 2004
25. Kaplan R, Psaila B, Lyden D: Niche-to-niche migration of bone-marrow-derived cells. *Trends Mol Med* 13: 72–81, 2007
26. Sbaa E, De Wever J, Martinive P: Caveolin plays a central role in endothelial progenitor cell mobilization and homing in SDF-1-driven postischemic vasculogenesis. *Circ Res* 98: 1219–1227, 2006
27. Gill M, Dias S, Hattori K, Rivera M, Hicklin D, Witte L, Girardi L, Yurt R, Himel H, Rafii S: Vascular trauma induces rapid but transient mobilization of VEGFR2(+)AC133(+) endothelial precursor cells. *Circ Res* 88: 167–174, 2001
28. Heeschen C, Aicher A, Lehmann R, Fichtlscherer S, Vasa M, Urbich C, Mildner-Rihm C, Martin H, Zeiher A, Dimmeler S: Erythropoietin is a potent physiologic stimulus for endothelial progenitor cell mobilization. *Blood* 102: 1340–1346, 2003
29. Hattori K, Dias S, Heissig B, Hackett N, Lyden D, Tateno M, Hicklin D, Zhu Z, Witte L, Crystal R, Moore M, Rafii S: Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. *J Exp Med* 193: 1005–1014, 2001
30. Hattori K, Heissig B, Wu Y, Dias S, Tejada R, Ferris B, Hicklin D, Zhu Z, Bohlen P, Witte L, Hendrikx J, Hackett N, Crystal R, Moore M, Werb Z, Lyden D, Rafii S: Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone marrow microenvironment. *Nat Med* 8: 841–849, 2002
31. Ceradini D, Kulkarni A, Callaghan M, Tepper O, Bastidas N, Kleinman M, Capla J, Galiano R, Levine J, Gurtner G: Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 10: 858–864, 2004
32. Rabelink T, deBoer H, de Koning E, van Zonneveld A: Endothelial progenitor cells: More than an inflammatory response? *Arterioscler Thromb Vasc Biol* 24: 834–838, 2004
33. Hristov M, Erl W, Linder S, Weber P: Apoptotic bodies from endothelial cells enhance the number and initiate the differentiation of human endothelial progenitor cells *in vitro*. *Blood* 104: 2761–2766, 2004
34. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM: Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 85: 221–228, 1999
35. Adams V, Lenk K, Linke A, Lenz D, Erbs S, Sandri M, Tarnok A, Gielen S, Emmrich F, Schuler G, Hambrecht R: Increase of circulating endothelial progenitor cells in patients with coronary artery disease after exercise-induced ischemia. *Arterioscler Thromb Vasc Biol* 24: 684–690, 2004
36. Lambiase PD, Edwards RJ, Anthopoulos P, Rahman S, Meng YG, Bucknall CA, Redwood SR, Pearson JD, Marber MS: Circulating humoral factors and endothelial progenitor cells in patients with differing coronary collateral support. *Circulation* 109: 2986–2992, 2004
37. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T: Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 348: 593–600, 2003
38. Dimmeler S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, Rutten H, Fischtlscherer S, Martin H, Zeiher A: HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI-3-kinase/

Akt pathway. *J Clin Invest* 108: 391–397, 2001
39. Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M, Isner J: VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J* 18: 3964–3972, 1999
40. Bahlmann F, Spandau J, Landry A, Hertel B, Duckert T, Boehm S, Menne J, Heller H, Fliser D: Erythropoietin regulates endothelial progenitor cells. *Blood* 103: 921–926, 2004
41. Min T, Zhu C, Xiang W, Hui Z, Peng S: Improvement in endothelial progenitor cells from peripheral blood by ramipril therapy in patients with stable coronary artery disease. *Cardiovasc Drugs Ther* 18: 203–209, 2004
42. Iwakura A, Luedemenn C, Shastry S, Hanly A, Kearney M, Aiakawa R, Isner J, Asahara T, Losordo D: Estrogen-mediated, eNOS-dependent mobilization of bone marrow-derived endothelial progenitor cells contributes to reendothelialization after arterial injury. *Circulation* 108: 3115–3121, 2003
43. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Uhling C, Technau-Ihling K, Zeiher A, Dimmeler S: Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. *Nat Med* 9: 1370–1376, 2003
44. Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Stern D, Naritomi H, Matsuyama T: Administration of CD34+ cells after stroke enhances neurogenesis *via* angiogenesis in a mouse model. *J Clin Invest* 114: 330–338, 2004
45. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner J, Asahara T: Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med* 5: 434–438, 1999
46. Shintani S, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, Sasaki K, Shimada T, Oike Y, Imaizumi T: Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation* 103: 2776–2779, 2001
47. Patschan D, Patschan S, Chintala S, Goligorsky MS: Uric acid heralds ischemic tissue injury to mobilize endothelial progenitor cells. *J Am Soc Nephrol* 18: 1516–1524, 2007
48. Kuo M, Patschan D, Patschan S, Addabbo F, Goligorsky MS: Ischemia-induced exocytosis of Weibel-Palade bodies mobilizes stem cells. *J Am Soc Nephrol* 19: 2321–2330, 2008
49. Oostendorp R, Dormer P: VLA4-mediated interactions between normal human hematopoietic progenitors and stromal cells. *Leuk Lymphoma* 24: 423–435, 1997
50. Scott L, Priestley G, Papayanopoulou T: Deletion of α4-integrins from adult hematopoietic cells reveals roles in homeostasis, regeneration and homing. *Mol Cell Biol* 23: 9349–9360, 2003
51. Papayanopoulou T: Current mechanistic scenarios in hematopoietic stem/progenitor cell mobilization. *Blood* 103: 1580–1585, 2004
52. Hu Y, Davison F, Zhang Z, Xu Q: Endothelial replacement and angiogenesis in arteriosclerotic lesions of allografts are contributed by circulating progenitor cells. *Circulation* 108: 3122–3127, 2003
53. Schroeter M, Leifheit M, Sudholt P: Leptin enhances the recruitment of endothelial progenitor cells into neointimal lesions after vascular injury by promoting integrin-mediated adhesion. *Circ Res* 103: 536–544, 2008
54. Kucia M, Reca R, Miekus K, Wanzeck J, Wojakowski W, Janowska-Wieczorek A, Ratajczak J, Ratajczak MZ: Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: Pivotal role of SDF-1-CXCR4 axis. *Stem Cells* 23: 879–894, 2005
55. Penn M, Mangi A: Genetic enhancement of stem cell engraftment, survival, and efficacy. *Circ Res* 102: 1471–1482, 2008
56. Togel F, Isaac J, Hu Z, Weiss K, Westenfelder C: Renal SDF-1 signals mobilization and homing of CXCR4-positive cells to the kidney after ischemic injury. *Kidney Int* 67: 1772–1784, 2005
57. Napoli C, Balestrieri A, Ignarro L: Therapeutic approaches in vascular repair induced by adult bone marrow cells and circulating progenitor endothelial cells. *Curr Pharm Design* 13: 3245–3251, 2007
58. Moore K, Lemischka I: Stem cells and their niches. *Science* 311: 1880–1885, 2006
59. Tanaka K, Sata M, Natori T, Kim-Kaneyama J, Nose K, Shibanuma M, Hirata Y, Nagai R: Circulating progenitor cells contribute to neointimal formation in nonirradiated chimeric mice. *FASEB J* 22: 428–436, 2008
60. Loomans C, van Haperen R, Duijs J, Verseyden C, de Crom R, Leenen P, Drexhage H, de Boer H, de Koning E, Rabelink T, Staal F, van Zonneveld A: Differentiation of bone marrow-derived endothelial progenitor cells is shifted into a proinflammatory phenotype by hyperglycemia. *Mol Med* 15: 152–159, 2009
61. Tian F, Liang P, Li L: Inhibition of endothelial progenitor cell differentiation by VEGI. *Blood* 113: 5352–5360, 2009
62. Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K, Ohmura M, Naka K, Hosokawa K, Ikeda Y, Suda T: Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat Med* 12: 446–451, 2006
63. Tepper O, Galiano R, Capla J, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC: Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion and incorporation into vascular structures. *Circulation* 106: 2781–2786, 2002
64. Kong D, Melo L, Mangi A, Zhang L, Lopez-Ilasaca M, Perrella MA, Liew CC, Pratt RE, Dzau VJ: Enhanced inhibition of neointimal hyperplasia by genetically engineered endothelial progenitor cells. *Circulation* 109: 1769–1775, 2004
65. Murasawa S, Llevadot J, Silver M, Isner J, Losordo D, Asahara T: Constitutive human telomerase reverse-transcriptase expression enhances regenerative properties of endothelial progenitor cells. *Circulation* 106: 1133–1139, 2002
66. Liu J, Dunoyer-Geindre S, Blot-Chabaud M, Sabatier F, Fish R, Bounameaux H, Dignat-George F, Kruithof E: Generation of human inflammation-resistant endothelial progenitor cells by A20 gene transfer. *J Vasc Res* 47: 157–167, 2009
67. Di Stefano V, Cencioni C, Zaccagnini G, Magenta A, Capogrossi M, Martelli F: p66ShcA modulates oxidative stress and survival of endothelial progenitor cells in response to high glucose. *Cardiovasc Res* 82: 421–429, 2009
68. Seeger F, Chen L, Spyridopoulos I, Altschmied J, Aicher A, Haendeler J: Down-regulation of ETS rescues diabetes-induced reduction of endothelial progenitor cells. *PLoS One* 4: e4529, 2009
69. Yamamizu K, Kawasaki K, Katayama S, Watabe T, Yamashita J: Enhancement of vascular progenitor potential by protein kinase A through dual induction of Flk-1 and neuropilin-1. *Blood* 114: 3707–3716, 2009
70. Xu Q: Progenitor cells in vascular repair. *Curr Opin Lipidol* 18: 534–539, 2007
71. Lapergue B, Mohammad A, Shuaib A: Endothelial progenitor cells and cerebrovascular diseases. *Prog Neurobiol* 83: 349–362, 2007
72. Povsic T, Goldschmidt-Clermont P: Endothelial progenitor cells: Markers of vascular reparative capacity. *Therapeutic Adv Cardiovasc Dis* 2: 199–213, 2008
73. Schatterman G, Hanlon H, Dobbs S, Christy B: Blood-derived angioblasts accelerate blood flow restoration in diabetic mice. *J Clin Invest* 106: 571–578, 2000
74. Langer H, May A, Daub K, Heinzmann U, Lang P, Schumm M, Vestweber D, Massberg S, Schönberger T, Pfisterer I, Hatzopoulos AK, Gawaz M: Adherent platelets recruit and induce differentiation of murine embryonic endothelial progenitor cells to mature endothelial cells in vitro. *Circ Res* 98: e2–e10, 2006
75. Young P, Vaughan D, Hatzopoulos A: Biological properties of endothelial progenitor cells and their potential for cell therapy. *Prog Cardiovasc Dis* 49: 421–429, 2007
76. Perry T, Song M, Despres D, Kim S, San H, Yu Z, Raghavachari N, Schnermann J, Cannon R, Orlic D: Bone marrow-derived cells

do not repair endothelium in a mouse model of chronic endothelial cell dysfunction. *Cardiovasc Res* 84: 317–325, 2009
77. Tsuzuki M: Bone marrow-derived cells are not involved in reendothelialized endothelium as endothelial cells after simple endothelial denudation in mice. *Basic Res Cardiol* 104: 601–611, 2009
78. Pula G, Mayr U, Evans C, Prokopi M, Vara D, Yin X, Astroulakis Z, Xiao Q, Hill J, Xu Q, Mayr M: Proteomics identifies thymidine phosphorylase as a key regulator of the angiogenic potential of colony-forming units and endothelial progenitor cells in culture. *Circ Res* 104: 32–40, 2009
79. Li L, Truong P, Igarashi P, Lin F: Renal and bone marrow cells fuse after renal ischemic injury. *J Am Soc Nephrol* 18: 3067–3077, 2007
80. Rustom A, Saffrich R, Markovic I, Walther P, Gerdes H-H: Nanotubular highways for intercellular organelle transport. *Science* 303: 1007–1010, 2004
81. Spees J, Olson S, Whitney M, Prockop D: Mitochondrial transfer between cells can rescue aerobic respiration. *Proc Natl Acad Sci U S A* 103: 1283–1288, 2006
82. Gurke S, Barroso J, Gerdes H-H: The art of cellular communication: Tunneling nanotubes bridge the divide. *Histochem Cell Biol* 129: 539–550, 2008
83. Leach K: Multifunctional cell-instructive materials for tissue regeneration. *Regen Med* 1: 447–455, 2006
84. Gerecht S, Burdick J, Ferreira L, Townsend A, Langer R, Vunjak-Novakovic G: Hyaluronic acid hydrogel for controlled self-renewal and differentiation of human embryonic stem cells. *Proc Natl Acad Sci U S A* 104: 11298–11303, 2007
85. Cribbs S, Martin G, Rojas M: Monitoring of endothelial dysfunction in critically ill patients: The role of endothelial progenitor cells. *Curr Opin Crit Care* 14: 354–360, 2008
86. Krenning G, Dankers P, Drouven J, Waanders F, Franssen C, van Luyn M, Harmsen M, Popa E: Endothelial progenitor cell dysfunction in patients with progressive chronic kidney disease. *Am J Physiol Renal* 296: F1314–F1322, 2009
87. Jourde-Chiche N, Dou L, Sabatier F, Calaf R, Cerini C, Robert S, Camoin-Jau L, Charpiot P, Argiles A, Dignat-George F, Brunet P: Levels of circulating endothelial progenitor cells are related to uremic toxins and vascular injury in hemodialysis patients. *J Thromb Haemost* 7: 1576–1584, 2009