Stem/Progenitor Cells in Vascular Regeneration

Li Zhang, Qingbo Xu

Stem cell therapy could be a promising option for the treatment of vascular diseases. The finding of stem/progenitor cells in circulating blood and the vessel wall indicates that endogenous (adult) stem cells have the ability to repair endothelial cells, thereby restoring the integrity of the vessel and most importantly its function.1 However, embryonic stem cells, which are a population of undifferentiated cells that can develop into any type of cells in the body including endothelial and smooth muscle cells, occur as an another source for stem cell therapy.2 Finally, recent progress in somatic cell reprogramming (ie, induced pluripotent stem [iPS] cells), propelling the research field toward more attractive and promising heights.3 Overall, significant progress in stem cell research has been made recently, especially the mechanisms of stem cell activation, homing, and differentiation in vascular repair and remodeling.4 In this regard, many recent publications in ATVB have provided further insight into the functional role of stem/progenitor cells in (1) rescuing ischemic tissues,5–10 (2) the benefits of progenitor cells in endothelial regeneration and smooth muscle accumulation in neointima, and (3) the stem cell differentiation into vascular lineages.11–14 In the present article, we will highlight these recent publications within a brief context of the literature on vascular stem/progenitor cells.

Circulating Proangiogenic Cells

Since the first report on endothelial progenitor cells in blood in 1997,15 a large number of articles in the field have been published, in which a large proportion was describing early endothelial progenitor cells and colony-forming cells.16–18 Most of these cells were identified to be monocytes/macrophages.16–18 It is now conceivable that a small population of outgrowth cells cultivated for several passages from human blood could be late endothelial progenitor cells.19 Although the nature and functions of endothelial progenitors are controversial, it is conceivable that a population of blood cells plays a role in promoting angiogenesis or endothelial repair.19 Several experimental studies have demonstrated that proangiogenic cells from either the bone marrow or circulating blood are essential for functional recovery of ischemic tissue after cell therapy.20,21 Vascular disease risk factors, such as oxidative stress, can interrupt the functions of proangiogenic cells, resulting in delayed functional recovery of ischemic tissues.22 The concentration of high-density lipoprotein has shown an influence on the number of proangiogenic cells in blood, which is related to the function of human proangiogenic cells and angiogenesis by activating Rho-associated kinase pathways.23 Interestingly, a recent report demonstrated that hypoxic preconditioning can expand old endothelial progenitors without senescence through modulation of the hypoxia-induced hypoxia-inducible factor-1α-TWIST-p21 axis.24

CD34 is a 105- to 120-kDa cell-surface glycoprotein that functions as a cell-to-cell adhesion factor selectively expressed on stem, progenitor, and vascular endothelial cells within hematopoietic systems.25 The expanded CD34+ cells are shown to be useful for therapeutic angiogenesis in ischemic heart.26 Clinical trials for CD34+ cell therapy for ischemic disease have been performed in several research centers.27–29 Although the mechanisms on how CD34+ cells exert their role in angiogenesis are uncertain, a recent article indicated that CD34 hybrid cells promote endothelial colony-forming cell bioactivity and have a therapeutic potential for ischemic diseases.30 Furthermore, several groups have studied the effects of genes and microenvironment on the functions of proangiogenic or endothelial progenitor cells in vivo and in vitro.31,32 A variety of studies support the role of proangiogenic cells in endothelial regeneration. However, a golden standard for endothelial progenitor cell isolation, characterization, and functions is needed for future investigation.

Vascular Resident Stem Cells

It was found that all 3 layers of the vessels, the intima, media, and adventitia, contain resident progenitor cells, including endothelial progenitor cells, mesenchymal stromal cells, Sca-1+ and CD34+ cells.33 In the pathogenesis of vascular disease, stem/progenitor cells may participate in vascular repair and the formation of neointimal lesions in severely damaged vessels.34 In the intima, it has been demonstrated that endothelial progenitor cells are resident within the intima of umbilical vein and aortic vessel wall and can contribute to endothelial formation.17 These endothelial progenitor/stem-like cell populations in the intima have the potential to form neovessels and are able to produce large numbers of endothelial cells in vitro and in vivo.34 In the arterial media of the mice, a current report by Tang et al35 identified a new type of stem cells in the blood vessel wall, named multipotent vascular stem cells, which differentiate into neural cells and mesenchymal stem cell–like cells that can subsequently differentiate into smooth muscle cells. In the adventitia, abundant stem/progenitor cells expressing Sca-1 have been identified, which contribute to endothelial regeneration and smooth muscle accumulation in the neointimal lesions.36 These results indicate the complexity of vascular stem cells, which could be closely related to vascular repair and disease development4 (Figure 1).
Inflammatory response in the intima of the artery leads to the release of cytokines,\(^\text{37}\) of which stromal cell–derived factor-1 (also known as chemokine [C-X-C motif] ligand 12 [CXCL12]) is a main chemokine for recruitment of CXC chemokine receptor type 4 (CXCR4)–positive stem/progenitor cells.\(^\text{38}\) In the pathogenesis of atherosclerosis, stromal cell–derived factor-1 expression in the intimal lesions was upregulated, and CXCR4+ stem cells migrated into the intima via interaction with inflammatory endothelium.\(^\text{39}\) It was shown that in vivo neutralization of stromal cell–derived factor-1 inhibited stem cell homing.\(^\text{40}\) A study using ex vivo model demonstrated that stromal cell–derived factor-1 can attract vascular stem cells strongly from the adventitial side migrating to the intima where they differentiated into vascular cells.\(^\text{41}\) Once stem cells are attracted by chemokines to the activated/inflammatory endothelium, these cells may differentiate into a variety of cell lineages depending on the factors in the microenvironment.\(^\text{42,43}\) Accumulating evidence indicates that the mobilization and recruitment of tissue resident stem/progenitor cells giving rise to endothelial and smooth muscle cells may participate in atherosclerosis.\(^\text{19,44}\)

Drug-eluting stents inhibiting smooth muscle proliferation (eg, sirolimus) are routinely used for the treatment of patients with coronary artery occlusion. In the long term, delayed endothelial healing and thrombus formation in the stented artery often occur.\(^\text{45}\) A recent report implicated that sirolimus induces stem/progenitor cell migration and differentiation into smooth muscle cells via CXCR4 and epidermal growth factor receptor/extracellular signal–regulated kinase/β-catenin signal pathways, thus indicating a novel mechanism of restenosis formation after sirolimus-eluting stent treatment.\(^\text{46}\) At this point, vascular resident stem/progenitors have a role to expedite lesion formation during restenosis although they may serve to promote atherosclerotic plaque stabilization by producing extracellular matrix proteins in less inflammatory intima. This provides profound evidence for the hypothesis that vascular stem cells and progenitors may act as a double-edged sword in the pathogenesis of restenosis, depending on the drug used for stent coating.\(^\text{4}\)

How do stem/progenitor cells decide the direction for their differentiation either to endothelium or smooth muscles? At the present, this remains unclear. However, inflammatory responses mediated by macrophages could be crucial. A recent article demonstrating that macrophages control vascular stem/progenitor cell plasticity through tumor necrosis factor–α–mediated nuclear factor–κB activation\(^\text{47}\) supports this hypothesis. In vitro study suggests that macrophages can induce endothelial differentiation of the stem/progenitor cells, while simultaneously inhibiting their smooth muscle cell differentiation. Mechanistically, both effects were mediated by macrophage-derived tumor necrosis factor–α via tumor necrosis factor–α receptor I and canonical nuclear factor–κB activation.\(^\text{47}\) These results highlight the role of macrophages in directing vascular stem/progenitor cell lineage commitment. For future studies, it is essential to understand the regulatory networks that control endothelial and smooth muscle progenitor differentiation, which is undoubtedly fundamental for both basic research and for improving current therapeutic avenues for vascular diseases. It is thus necessary to study how other inflammatory cytokines influence vascular cell differentiation.

**Mesenchymal Stem Cells**

Mesenchymal stem cells are multipotent stromal cells that can differentiate into a variety of cell types, including smooth muscle cells, osteoblasts, chondrocytes, and adipocytes.\(^\text{46}\) Mesenchymal stem cells can be isolated from the internal surface of human saphenous vein and cultivated in vitro.\(^\text{48}\) They form a semiconfluent layer of spindle-shaped cells that are CD13+, CD29+, CD44+, CD34−, CD45−, CD54+, CD106−, CD90+, KDR−, cadherin-5−, HLA class I+, and HLA-DR−. Gene expression analysis revealed that the cells express mesenchymal stem cell markers and can differentiate in a similar pattern to bone marrow–derived mesenchymal stem cells.\(^\text{49}\) Thus, these stem cells were found in both the vessel wall and the bone marrow.

As mentioned above, inflammation is a hallmark of several types of vascular diseases, in which macrophages play a central role. A recently published article demonstrated that macrophages induce differentiation of human adipose tissue–derived mesenchymal stem cells to α-actin+ smooth muscle cells, displaying a contractility in response to KCl and carbachol treatment.\(^\text{50}\) Transplantation of the differentiated smooth muscle cells attenuated severe hindlimb ischemia and promoted vascular regeneration through a prostaglandin F\(_{2\alpha}\)–mediated paracrine mechanism.\(^\text{50}\) However, monocyte chemotactic protein-1 mediated migration of mesenchymal stem cells that serve as a source of intimal hyperplasia.\(^\text{51}\) Transplantation experiments with labeled cells demonstrated that the adventitia served as a major source of cells migrating to the intima, in which monocyte chemotactic protein-1 is a potent chemokine for the recruitment of stem/progenitor cells to the lesions.\(^\text{51}\)
In the late stage of atherosclerosis, plaque rupture is a major clinical complication in patients. To stabilize the plaque, some investigators explored the possibility of cell therapy via infusion of mesenchymal stem cells or smooth muscle progenitors. Allogeneic bone marrow mesenchymal stem cell transplantation showed an important role in the stabilization and repair of ruptured atherosclerotic plaque. Furthermore, matrix metalloproteinase-8 expressed in stem/progenitor cells plays a part in mediating cell migration and their recruitment into atherosclerotic lesions. The number of stem/progenitor cells in atheroma was significantly reduced by genetic deletion of matrix metalloproteinase-8 in apolipoprotein E–deficient mice fed a Western diet. This is related to lesion development and stability. Interestingly, CXCL12 promotes the mobilization and neointimal recruitment of smooth muscle progenitor cells. Intravenous injection of CXCL12 selectively increased the level of Sca-1+/Lin−/platelet-derived growth factor receptor-β+ progenitor cells in the circulation. Silencing of arterial CXCL12 expression during atherosclerosis promoted lesion formation and reduced the lesional smooth muscle cell content in CXCL12-treated mice. Thus, CXCL12-induced progenitor mobilization seems a promising approach to treat unstable atheroma. Recently, endothelial CXCR4 deficiency was found to exacerbate neointima formation after arterial injury in atherosclerosis-prone mice. This was associated with lesion inflammation, endothelial cell proliferation, and reduced mobilization of Sca1+Lin− and Sca1+Flk1+CD31+ cells, often referred to as circulating endothelial progenitor cells, which could be related to a reduction in local CXCL12 expression in mice with endothelial CXCR4 deficiency. These data further highlight important and cell-specific regulatory functions of the CXCL12/CXCR4 axis in vascular homeostasis and repair under atherogenic conditions.

Endothelial regeneration and angiogenesis are key events for the restoration of blood supply to ischemic tissues. Transplantation of mesenchymal stem cells protects the heart against ischemia-reperfusion injury. Because evidence suggests a vital role of aldehyde dehydrogenase-2 in microenvironment homeostasis after ischemia, local overexpression of aldehyde dehydrogenase-2 significantly magnified mesenchymal stem cells–induced improvement of blood supply. Aldehyde dehydrogenase-2 could be a key mediator of host stem cell niche for optimal stem cell therapy. Furthermore, perivascular delivery of encapsulated mesenchymal stem cells improves posts ischemic angiogenesis via paracrine activation of vascular endothelial growth factor-A. Thus, mesenchymal stem cell therapy is a promising treatment for ischemic injury as well.

Embryonic Stem Cells/iPS Cells

Embryonic stem cells are a valuable source of pluripotent stem cells with unlimited growth and self-renewal abilities and are able to differentiate into vascular lineage in vitro and in vivo. Similarly, iPS cells derived from somatic cell population have an ability to differentiate into almost all types of tissues. A recent study aimed at understanding the molecular mechanisms of iPS cell differentiation into endothelial cells that can be used to promote angiogenesis. Meanwhile, both embryonic stem and iPS cells were successfully differentiated into smooth muscle cells that were useful for generation of tissue-engineered vessels.

Several methods for endothelial differentiation from stem cells have been described, in which a protocol using collagen IV–coated plate and vascular endothelial growth factor stimulation to drive the functional differentiation of iPS cells into endothelial cells was used. It was found that micro-RNA-21 and transforming growth factor-β2 signaling pathways mediated the vascular endothelial growth factor–induced endothelial lineage differentiation of iPS cells. Furthermore, Orlova et al developed defined conditions for simultaneous derivation of endothelial cells and pericytes with high efficiency from human iPS cells of different tissue origin. It was showed that human iPS cell–derived endothelial cells integrated into developing vasculature as xenografts in zebrafish, indicating that iPS-derived cells are fully functional and can be used to study defective endothelium–pericyte interactions in vitro for disease modeling and studies on angiogenesis. Using embryonic stem cell–derived endothelial cells, the improvement in blood flow of ischemic tissues was observed in animal models. Interestingly, a clinical-grade human neural stem cell line promotes angiogenesis and neurogenesis in a preclinical model of stroke and is now showed the therapeutic activity of intramuscular implantation in murine models of hindlimb ischemia. In addition, mice iPS cell–derived Flk-1+ or Flk-1− cells were intravenously injected into the mice after vascular injury that significantly attenuated neointimal hyperplasia when compared with controls. Administration of iPS cell–derived Flk-1+ cells significantly enhanced reendothelialization when compared with that of the Flk-1− cell control group, indicating a potentially useful therapeutic means for vascular dysfunction and prevention of restenosis after angioplasty.

Vascular smooth muscle cells were also successfully derived from embryonic stem cells using different culture conditions. One method is to cultivate embryonic stem cells on collagen IV–coated plates plus transforming growth factor-β/platelet-derived growth factor stimulation. It was found that phospholipase A2, group 7 (Pla2g7) is an important mediator for stem cell differentiation into smooth muscle cells. Knockdown of Pla2g7 resulted in downregulation of smooth muscle–specific markers in vitro and impairment of smooth muscle differentiation in vivo, whereas enforced expression of Pla2g7 enhanced its differentiation and increased reactive oxygen species generation. Moreover, it was demonstrated that nuclear factor erythroid 2–related factor 3 regulates Pla2g7 gene expression through direct binding to the promoter regions of Pla2g7 gene. This study highlights a novel mechanism of stem cell differentiation into smooth muscle cells that could be useful for the creation of tissue-engineered vessels.

Direct Reprogramming of Vascular Cells

One of most exciting fields of iPS cell–related research is the direct conversion of somatic cells, such as fibroblasts, into other somatic cell fates. In cardiac research, it was shown that a combination of 3 developmental transcription factors, Gata4, Mef2c, and Tbx5, could rapidly and efficiently reprogram postnatal cardiac or dermal fibroblasts directly into differentiated cardiomyocyte-like cells. Margariti et al developed a method to generate partial-iPS cells by transferring 4
reprogramming factors (OCT4, SOX2, KLF4, and c-MYC) to human fibroblasts. Functionally, partial-iPS cells can differentiate into endothelial cells useful for the improvement of neo-vascularization and blood flow recovery in hindlimb ischemic model. Furthermore, partial-iPS–derived endothelial cells displayed good attachment, stabilization, patency, and typical vascular structure when seeded on decellularized vessel scaffolds.67 Concomitantly, Li et al68 reported that human neonatal fibroblasts were transduced with lentiviruses encoding Oct4 and Klf4 in the presence of soluble factors that promote the induction of an endothelial program. When these endothelial cells were injected into the ischemic limb of mice, the cells engrafted, increased capillary density, and enhanced tissue perfusion.68 Thus, transcriptional factors–induced reprogramming can be successfully applied for generating functional endothelial lineages for therapeutic applications.

In line with endothelial production, we designed a combined protocol of reprogramming and differentiation of human neonatal fibroblasts into smooth muscle cells.69 The partial-iPS cells can differentiate into smooth muscle cells, expressing a panel of smooth muscle markers when seeded on collagen IV–coated plates with platelet-derived growth factor–containing medium. Partial-iPS–derived smooth muscle cells repopulated decellularized vessel grafts and ultimately gave rise to functional tissue-engineered vessels when combined with endothelial cells. The grafts were surviving in immunodeficient mice after transplantation of the vessel.69 These findings provide a new insight into the mechanisms of smooth muscle differentiation with vast therapeutic potential.

Summary
A series of studies has been presented in the search for proof of circulating and resident vascular progenitor cells, which can differentiate into endothelial and smooth muscle cells and pericytes in animal and human studies.2,33 In terms of pluripotent stem cells, including embryonic stem cells, iPS, and partial-iPS cells, they display a great potential for vascular lineage differentiation.2 Development of stem cell therapy for treatment of vascular and ischemic diseases remains a major challenging research field. At the present, there is a clear expansion of research into mechanisms of stem cell differentiation into vascular lineages that are tested in animal models. Although there are several clinical trials ongoing that primarily focus on determining the benefits of stem cell transplantation in ischemic heart or peripheral ischemic tissues,27,29 intensive investigation for translational aspects of stem cell therapy would be needed. It is a hope that stem cell therapy for vascular diseases could be developed for clinic application in the future.

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