Vascular Regeneration by Stem/Progenitor Cells

Yao Xie,* Ye Fan,* Qingbo Xu

Cardiovascular disease is a major cause of death worldwide. Many cardiovascular events, such as stroke and coronary heart disease, strongly correlate with loss of blood supply, leading to loss of cell function, organ failure, and eventually death.[1] Vascular regeneration, which includes restoration of normal vascular structure and function, the reversal of vascular senescence, and the growth of new blood vessels may be a promising approach for the treatment of these diseases.[2] Stem/progenitor cells are able to differentiate into vascular cell lineages, which may contribute to the regenerative process and could be useful for the disease treatment.[3] Recently, several publications in *Arteriosclerosis, Thrombosis, and Vascular Biology* and other journals have demonstrated great progress in research into stem/progenitor cells for vascular regeneration.[4–6] In the present article, we will highlight these updated publications, providing insights into the role of stem/progenitor cells in restoration of vascular function under pathophysiological conditions and the contribution of these cells to lesion formation in the vessel wall through the mechanisms of cell migration and differentiation.

Circulating Progenitor Cells and Inflammation

Since the discovery of hemangiogenic cells in the bone marrow, researchers have been searching for other such pools of circulating progenitors, such as those that may contribute to vascular repair after ischemic injury. The study of circulating endothelial progenitors, termed endothelial progenitor cells (EPCs), became an extremely attractive field of research after they were first isolated by Asahara et al[7] in 1997, using magnetic bead selection based on expression of specific cell surface antigens. Though a large number of articles have since been published using the term EPCs,[8–10] its initial use was a misnomer because the surface markers used for cell isolation and characterization, such as vascular endothelial growth factor receptor 2 (VEGFR2) and CD133,[11] are now known not to be specific for endothelial lineages.[12] In spite of this, the term EPC still appears in the literature though there is great controversy about its relevance. Some populations of these cells have been shown to exert a strong paracrine mitogenic effect on mature endothelial cells via cytokine secretion and

to enhance the angiogenic response to hind limb ischemia through release of growth factors and sustained upregulation of recipient cytokines and growth factors.[13,14] However, in other reports, the contribution of EPCs to endothelial repair has been estimated to be low, if indeed there is any.[15–18] It is also questionable whether EPCs represent a defined circulating population or are in some cases merely a phenomenon of cell culture.[17] Although there are tens of thousands of publications focusing on circulating EPCs, a gold standard definition of the surface markers they should display and a standardized protocol for their isolation is still under debate.

One disease in which progenitors which can differentiate into endothelial cells have been proposed to play a role is diabetes mellitus.[8] Both clinical and basic scientific research indicates that this condition may alter the properties of endothelial progenitor cells, leading to variation in their contribution to endothelial repair and neovascularization at the site of vascular injury or ischemia.[19] Because of inflammation, oxidative stress, depletion of nitric oxide, impaired glucose metabolism, hyperglycemia, and insulin resistance in patients with diabetes mellitus, a reduction in number or a dysfunction in the progenitor cells could have a pathological effect on cell behavior in various processes, such as mobilization and differentiation in the bone marrow, migration and survival in blood, and the homing process.[19] Various approaches such as use of anti-diabetic agents, inhibition of dipeptidyl peptidase-4 (DPP4) and C-X-C chemokine receptor type 4 (CXCR-4), and other biological therapies have been found to be beneficial in restoring the progenitor cell–related repair in diabetes mellitus.[19] A recent study revealed that type 1 insulin–like growth factor receptor, which is a negative regulator of insulin sensitivity and nitric oxide availability, has a strong relationship with endothelial repair.[20] $Sca-1^+Flk-1^+$ circulating angiogenic progenitor cells in insulin-like growth factor receptor$^{+/−}$ mice displayed enhanced angiogenic capacity after wire injury. Partial deletion of insulin-like growth factor receptor in bone marrow–derived $CD117^+$ cells transfused into wild-type mice increased endothelial regeneration capacity without altering atherosclerosis, indicating a potential future strategy to enhance endothelial repair clinically.[20]

In recent years, several groups have reported the effects of various genes and proteins on the function, differentiation, and migration of circulating progenitor cells both in vivo and in vitro.[21,22] For instance, bone marrow protein 9, a member of the transforming growth factor-β family, has been indicated to contribute to the differentiation of the endothelial progenitor cells.[23] The expression of bone marrow protein 9 and its receptor, activin receptor–like kinase 1, was greatly altered during the differentiation of these progenitor cells. In addition, bone marrow protein 9 was found to induce neovascularization which could be blocked by inhibition of the activin receptor–like kinase 1 signaling pathway.[23] Another research group has

From the Cardiovascular Division, King's College London BHF Centre, London, UK (Y.X., Q.X.); and Institute of Respiratory, Xinqiao Hospital, Third Military Medical University, Chongqing, China (Y.F.).

*These authors contributed equally.

Correspondence to Qingbo Xu, MD, PhD, Cardiovascular Division, King's BHF Centre, 125 Coldharbour Lane, London SE5 9NU, UK. E-mail qingbo.xu@kcl.ac.uk

shown that homocysteine can induce the senescence of endothelial and progenitor cells via telomerase inactivation and length shortening.[24] The underlying mechanism behind this was revealed to be cross talk between CCCTC-binding factor and SP1, which contributed to homocysteine-induced human telomerase reverse transcriptase (hTERT) DNA methylation.[24]

Recent microRNA studies reveal a significant role for these short noncoding RNA in vascular regeneration through influence on stem/progenitor cell functions.[25,26] MicroRNA-31 (miR-31) and miR-720 were found to be expressed at lower levels in circulating endothelial progenitor cells of patients with coronary artery disease.[27] The pathways regulating miR-31, miR-720, and vasohibin were found to be critical for the activation of endothelial progenitor cells, and their absence resulted in coronary artery disease. The circulating concentrations of miR-31, miR-720, and vasohibin could present promising prognosis biomarkers for cardiovascular diseases.[27]

In another study, miR-22 was found to be markedly upregulated during smooth muscle cell differentiation from stem/progenitor cells with the great reduction of methyl CpG–binding protein 2.[28] It was also found that miR-27b expression was decreased in bone marrow–derived angiogenic cells.[23] Treatment with a miR-27b mimetic improved bone marrow–derived angiogenic cell function, including proliferation, adhesion, tube formation, and delayed apoptosis, but did not affect migration. Elevated thrombospondin-1 protein in angiogenesis was suppressed on transfection with a miR-27b mimic. miR-27b improved topical cell therapy of diabetic skin wound closure. Normal bone marrow–derived angiogenic cell therapy with miR-27b inhibition demonstrated reduced efficacy in wound closure, perfusion, and capillary formation. Local miR-27b delivery partly improved wound healing in diabetic mice. All of these discoveries above could be potential approaches of therapies of vascular regeneration.[23]

Inflammatory cytokines are crucial for the development of vascular diseases, and their influence on stem/progenitor cells has recently been emphasized.[29] Stromal cell-derived factor 1 (SDF-1), also known as C-X-C motif chemokine 12 (CXCL12), which binds to receptor CXCR4, is a potent chemotactic to many types of cells, including endothelial progenitor cells.[30,31] It was believed to act as a novel suppressor of endothelial permeability by activating the CXCR4/phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Ras-related C3 botulinum toxin substrate 1 (RAC1) signaling pathway in endothelial cells.[32] Specific deletion of endothelial CXCR4 in a CRE mouse model led to a reduction in number of Sca-1+Flk1+CD31+ endothelial progenitor cells in the circulation with enhanced neointima formation 3 weeks after guidewire injury, indicating that CXCR4 is crucial for efficient reendothelialization.[33] However, a clinical study involving 3359 participants with 9.3 years of follow up revealed that SDF-1 was associated with heart failure and all-cause mortality but not with coronary artery disease or myocardial infarction.[34] It is well known that the cytokine transforming growth factor-α (TNF-α), released by macrophages, induces the endothelial inflammatory response by inducing expression of adhesion molecules.[35] A novel finding of the effect of TNF-α released by macrophages on stem/progenitor cells has recently been

reported.[36] It was demonstrated that TNF-α-mediated stem cell differentiation toward endothelial lineages was driven by direct binding of nuclear factor-κB (p65) to specific vascular endothelial cadherin (VE-cadherin) promoter sequences. TNF-α knockout in a mouse model of vein graft decreased re-endothelialization and significantly increased thrombosis formation. This study highlights the role of macrophages in directing vascular stem/progenitor cell lineage commitment through TNF-α-mediated TNF-α receptor 1 and nuclear factor-κB activation that is likely required for endothelial repair in vascular diseases.[36]

Vascular Resident Stem/Progenitor Cells

The presence of vascular resident stem cells has been determined in all 3 layers of the blood vessel wall, the intima, media, and adventitia (Figure 1).[37] Investigation into murine vessels reveals that Sca-1+ progenitor cells exist in the adventitia of large- and medium-sized vessels, which are able to migrate into the intima when seeded on the adventitial surface in matrigel.[38] Similarly, Sca-1+/Lin−/c-kit−/CD34− cells were found in the media of mouse thoracic/abdominal aorta and were shown to differentiate into endothelial cells or smooth muscle cells in response to vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF) in culture medium.[39] In addition, the intima was also proven to have endothelial progenitor cells which could contribute to vascular regeneration.[40,41] The ratio between endothelial and smooth muscle cells remains stable under normal physiological conditions but is altered markedly in the pathogenesis of vascular diseases.
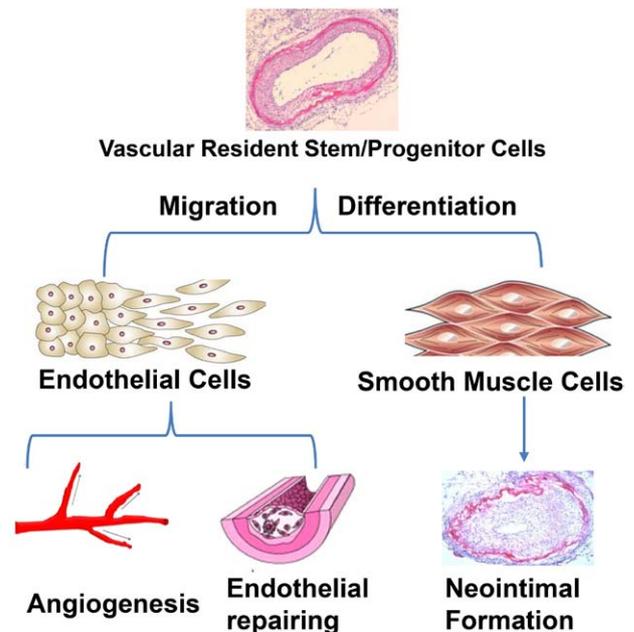

Figure 1. Vascular resident stem/progenitor cells were discovered in all 3 layers of vessels. In injured blood vessels, vascular stem/progenitor cells are capable of migrating into the damaged area in response to various stimuli. The cells can then differentiate either into endothelial or smooth muscle cells, respectively, depending on the profile of different cytokines released. The migration and differentiation of vascular stem/progenitor cells contributes to angiogenesis, endothelial repair, and neointimal formation.

Accumulating evidence demonstrates that vascular stem/progenitor cells may contribute to this process and lead to vascular regeneration.[42] Furthermore, it was reported that there are resident macrophage progenitor cells in the adventitia of the mouse aorta. These Sca-1^{+}/CD45^{+} progenitor cells were not derived from circulation and were found at higher levels in a mouse model of atherosclerosis, which provides evidence that vascular resident progenitor cells are a heterogeneous population.[43]

Vascular resident stem/progenitor cells have a great potential in cardiovascular regeneration therapies.[42] In a recent study, adventitial progenitor cells were harvested from veins during coronary artery bypass graft.[44] These progenitors showed antioxidant properties with upregulation of antioxidant enzymes, such as superoxide dismutase and catalase, the effects of which can be abolished by pharmacological inhibition of superoxide dismutase. In a mouse limb ischemia model, injection of these progenitor cells induced neovascularization which offered protection from ischemia.[44] The role of human saphenous vein–derived adventitial progenitor cells was investigated in a similar mouse model of limb ischemia. Transplanted cells reached their therapeutic target and triggered revascularization of ischemic limbs. In addition, fms-related tyrosine kinase 1 (FLT-1) gene silencing in these progenitor cells markedly reduced their ability to form tubes, confirming the outcomes from predicted expressional studies.[45] Resveratrol is a natural phytochemical which was recently found to induce vascular stem/progenitor differentiation into endothelial cells, which express CD31 and endothelial nitric oxide synthase (eNOS) with the great reduction of miR-21, Akt phosphorylation, and nuclear β-catenin expression. Progenitor cells applied with resveratrol treatment demonstrated an enhanced ability for re-endothelialization ex vivo, and reduced lesion formation was seen with a resveratrol diet in a mouse model of vessel graft.[46]

Vascular disorder in the lung could be a lethal disease at the final stage, which is related to the loss of functional microvasculature.[47] Progenitor cells had been found in lungs adjacent to small vessels.[48] These cells can differentiate into smooth muscle cells in vitro. In vivo, an increased recruitment of these progenitors in response to chronic hypoxia was observed. Subsequently, these progenitors can differentiate into smooth muscle cells in the remodeled vessels, suggesting the participation of the progenitor cells in vascular remodeling.[48] Furthermore, resident endothelial cells and progenitors were capable of proliferation and restoring endothelial barrier function after inflammatory lung injury.[49] The suppression of endothelial and progenitor cell proliferation by blocking intrinsic nuclear factor-κB at the barrier repair phase was associated with an augmented endothelial permeability and impeded endothelial barrier recovery.[49] In lungs, 8 weeks after injury, both endothelial and progenitor cells contribute to endothelial repair for endothelial barrier restoration.

Experiments in large animal models are of great value where possible. One group succeed to isolate a population of CD44^{+}CD56^{+}CD90^{+}CD105^{+}CD34^{−}CD45^{−} progenitor cells from porcine aorta, which was shown to be useful for vascular regeneration in the pig model.[50] Taken together, the current findings demonstrate a great potential to use resident stem cells for vascular regeneration and angiogenesis. The mechanism of stem/progenitor cell differentiation into endothelial or smooth muscle cells may involve in the alteration of biomechanical stimuli, that is, shear stress and cyclic strain.[51] Accumulating evidence indicates that laminar shear stress and cyclic strain could induce the differentiation of stem/progenitor cells into endothelial or smooth muscle cells, respectively. Further details of the mechanisms behind this have been reviewed elsewhere.[52]

Mesenchymal Stem Cells and Hematopoietic Stem Cells

Mesenchymal stem cells are a group of multipotent stromal cells which can differentiate into various types of cells, such as osteoblasts, chondrocytes, myocytes, and adipocytes.[53] Mesenchymal stem cells can be isolated from several different tissues, including umbilical cord, bone marrow, and adipose tissue.[54] The properties of these cells vary from tissue to tissue. For instance, cells isolated from adult bone marrow display a stable phenotype, expressing SH2, SH3, CD29, CD44, CD71, CD90, CD106, CD120a, CD124, and many other surface proteins and are capable of differentiation into adipocytic, chondrocytic, or osteocytic lineages.[55] Understanding the mechanisms behind cell differentiation, migration, mobilization, and homing will be a great help in the future clinical application of these cells as treatments for vascular diseases.

The poor retention of mesenchymal stem cells is a major obstacle to therapeutic utilization in ischemic tissues. To improve their retention and survival, researchers performed a series of studies and found that aldehyde dehydrogenase-2 (ALDH2) plays an important role in microenvironment homeostasis after ischemia.[56,57] One million green fluorescent protein (GFP) mesenchymal stem cells were transplanted into mice where limb ischemia model had been achieved by the ligation of femoral artery, and ALDH2-deficient tissue markedly reduced stem cell retention, blood perfusion recovery, and limb salvage effects after vascular injury. Moreover, overexpression of ALDH2 greatly enhanced mesenchymal stem cell survival via increased capillary density, oxidative stress regulation, and energy supply after ischemia, indicating that ALDH2 is a key enzyme for stem cell therapy.[56] Evidence also indicates that ALDH2 deletion significantly reduces blood perfusion recovery and increases smooth muscle cell atrophy. However, this process was mainly achieved by reduced proliferation, migration, and hypoxia-triggered tube formation of endothelial cells rather than mesenchymal stem cells. There was no significant improvement of blood flow to ischemic limbs when wild-type bone marrow was transplanted into ALDH2 knockout mice.[58] These opposing results from 2 groups demonstrate the complex role of ALDH2, and further investigation into its actions is needed. In addition, preactivating cellular glycogenesis by applying sublethal hypoxic preconditioning in mesenchymal stem cells, followed by a transplantation in ischemic thigh muscle in mouse, revealed a great improvement of limb salvage, blood perfusion recovery, and angiogenesis in ischemic tissues. Moreover, inhibition of glycogen synthesis abolished these improvements, providing a novel strategy of stem cell therapy.[57]
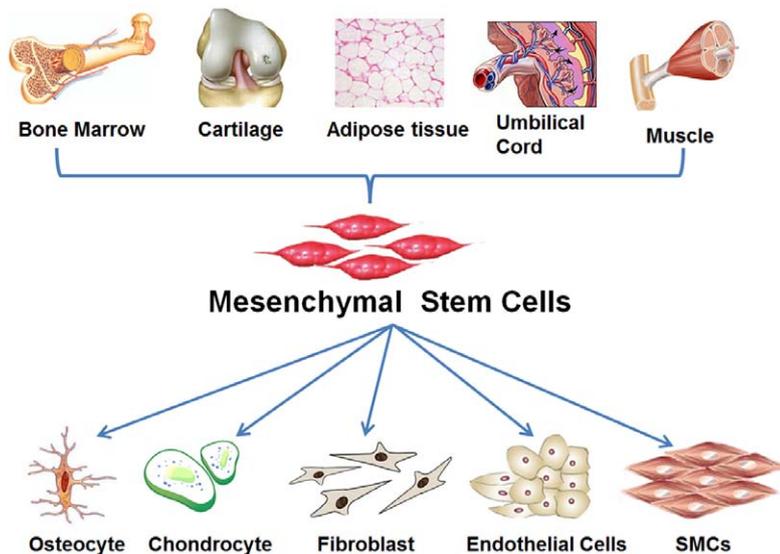
Figure 2. Mesenchymal stem cells can be isolated from various tissues, such as umbilical cord, bone marrow, cartilage, muscle, and adipose tissue. Cells from different tissues differ in properties including cell surface markers expressed and differentiation abilities. Mesenchymal stem cells can give rise to many cell lineages, such as osteocytes, chondrocytes, and fibroblasts. Under certain circumstances, mesenchymal stem cells in the circulation can migrate to an injured area, differentiating into endothelial cells to repair damage-triggering angiogenesis and smooth muscle cells (SMC) which potentially form neointimal lesions.

Mesenchymal stem cells can be derived from various sources and could be isolated from bone marrow, circulating blood, vascular wall, and adipose tissue (Figure 2).[59] Because of their easy isolation and relative abundance compared with other kinds of mesenchymal stem cells, adipose tissue stromal cells have recently become a popular cell type for stem cell research.[60] Injection of allogeneic abdominal adipose-derived stem cells, either with or without transfection with plasmid-VEGF165, has been shown to provide a protective role in a rabbit model of critical hind limb ischemia. These adipose-derived stem cells increased arteriolar density and protection against ischemia-induced muscle lesions.[61] CTX0E03, a line of human neural stem cells thought to promote angiogenesis and neurogenesis in stroke, also showed progress in a hind limb ischemia model.[62]

Though mesenchymal stem cells have a therapeutic benefit, recent studies suggest that they may also play a role in the pathology of aortic valve disease.[63] One potential mechanism for this is regulation of endothelial-to-mesenchymal transitions during stem cell differentiation. Deletion of Sox9 resulted in the calcification of aortic valve in the mouse model, illustrating a potential role of these progenitor cells in heart valve diseases, which may be explored in the development of future treatments.[63] It seems likely that further sources of mesenchymal stem cells exist.

Hematopoietic stem cells are a group of stem/progenitor cells which can give rise to both myeloid and lymphoid lineages of blood cells. They are found in the largest quantities in bone marrow but also exist in peripheral and umbilical cord blood. The physiological and pathological state of hematopoietic stem cells is vital for vascular regeneration.[64] ApoE$^{-/-}$ mice with monocytosis and atherosclerosis showed enhanced expansion of these cells associated with increased surface expression of common β subunit of granulocyte macrophage colony–stimulating factor/interleukin-3 receptor.[65] Transplantation of ApoE$^{-/-}$ bone marrow into Ldlr$^{-/-}$ mice resulted in an increased level of granulocyte macrophage colony–stimulating factor on stem cells and innate response activator B cells in spleen, which led to the expansion of stem cells. Therefore, granulocyte macrophage colony–stimulating factor could contribute to monocytosis and increased lesional macrophage content, affecting atherosclerosis plaque stabilization.[65] Scavenger receptor type BI, which is a highly density lipoprotein receptor, was found on murine hematopoietic stem cells. Scavenger receptor type B1 expression was related to the antiproliferative effects of high-density lipoprotein on hematopoietic stem cells. Scavenger receptor type BI$^{-/-}$ bone marrow–transplanted Ldlr$^{-/-}$ mice induced enhanced reconstruction of white blood cells, production of inflammatory cells, and plaque development. Moreover, high-density lipoprotein level was negatively correlated with the proliferation and differentiation of hematopoietic stem cells in the circulation, which was associated with atherosclerosis progression.[66] Another bone marrow–derived cell, megakaryocytes are responsible for platelet production. Recently, a group of scientists discovered that the ATP-binding cassette transporter B6 was highly expressed in megakaryocyte progenitors.[67] Deletion of the ATP-binding cassette transporter B6 resulted in expansion of megakaryocyte progenitors and increased platelet counts and platelet activity. Abcb6$^{-/-}$ bone marrow–transplanted mice expressed higher levels of C-C motif ligand 5 and accelerated atherosclerosis, leading to increased macrophage accumulation in atherosclerotic plaques.[67] Taken together, hematopoietic stem cells exert their effectiveness on atherogenesis via differentiation into macrophages/platelets or influencing lipid metabolisms.

Embryonic Stem Cells/Induced Pluripotent Stem Cells

Embryonic stem cells are pluripotent cells derived from the inner cell mass of the blastocyst, which have the ability to differentiate into any cell type and which can divide indefinitely to produce daughter cells in vitro and in vivo.[68] Various methods have been proposed for the differentiation of endothelial cells from embryonic stem cells, and the mechanisms behind this have been studied in great detail.[69] Bioengineered tissues are considered as a promising treatment in regenerative medicine. By performing 3-dimensional suspension culture and applying VEGF and 8-bromo-cAMP, stem cell–derived

CD31+ cells were successfully generated of and were able to form microvascular networks.[70] Co-culture of CD31+ stem cell–derived cells with cardiomyocytes and dermal fibroblasts led to tube formation in cardiac cell sheets, which could be useful for fabricating prevascularized cardiac cell sheet for grafting on to ischemic heart tissue or for preparation of a bioengineered heart on a scaffold.[70]

To understand the mechanisms behind stem cell differentiation into endothelial cells, Wu et al established a model to study epigenetic modification. They found that expression of histone demethylases KDM4A and KDM4C, which can bind to histones associated with Flk1 and VE-cadherin promoters, was upregulated during endothelial differentiation. Deletion of either KDM4A or KDM4C inhibited endothelial differentiation in murine embryonic stem cells in vitro and prevented blood vessel formation in zebrafish in vivo.[71]

In terms of smooth muscle cell differentiation from embryonic stem cells, there are a large number of reports into the mechanisms behind cell differentiation.[72] Recently, Wang et al[73] have reported that the expression of dickkopf homolog 3 (DKK3) is essential for the expression of smooth muscle markers and myocardin at both the mRNA and protein levels during stem cell differentiation into smooth muscle cells. Overexpression of DKK3 leads to further upregulation of the aforementioned markers. Further investigation indicates that DKK3 activates activating transcription factor 6, leading to the increased binding of activating transcription factor 6 to the myocardin promoter, increasing its expression.[73] These findings offer a novel mechanism by which DKK3 regulates stem cell differentiation by activating activating transcription factor 6 and promoting myocardin expression. In addition, expression of miR-22 was greatly enhanced during the differentiation of smooth muscle cells from stem cells.[28] Knockdown of miR-22 abolished this effect. In the meanwhile, methyl CpG–binding protein 2, which was the predicted target of miR-22, was also downregulated markedly. Further experiments demonstrated that miR-22 regulated smooth muscle cell differentiation via methyl CpG–binding protein 2 and its downstream activators.[28] The mechanism of embryonic stem cell differentiation into smooth muscle cells stimulated by DKK3 has been illustrated in Figure 3.

Differing from embryonic stem cells, induced pluripotent stem (iPS) cells are pluripotent stem cells which can be directly generated from adult cells. This technology was first developed by the Yamanaka laboratory in 2006 via the application of 4 specific gene encoding transcription factors, Oct4, Sox2, cMyc, and Klf4 in adult fibroblasts, which can be used to convert mature cells into pluripotent stem cells.[74]

iPS cells present a renewable source of endothelial and smooth muscle cells. It has recently been shown that low oxygen tension improves endothelial commitment and maturation during human pluripotent stem cell differentiation into early vascular cells.[75] Vascular cell differentiation in 5% oxygen gave rise to cells with an increased expression of endothelial cadherin and CD31 and arterial endothelial cell markers and capable of cord-like structure formation in a feeder-free, 2-dimensional differentiation system, indicating the importance of oxygen tension in human iPS cell differentiation.[75]
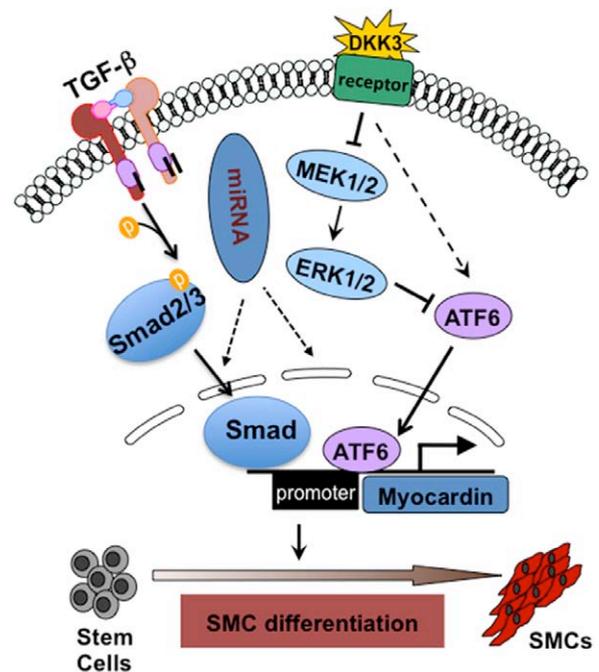

Figure 3. Schematic representation of the mechanism of stem cell differentiation toward smooth muscle cells. When stem cells are cultured in differentiation medium, they can differentiate into smooth muscle cells in response to dickkopf homolog 3 (DKK3) or transforming growth factor-β (TGF-β) stimulation. In this process, DKK3 is upregulated and secreted into the cell culture medium. miRNA exerts its role in gene expression. In turn, secreted DKK3 promotes the expression of activating transcription factor 6 (ATF6) via mitogen-activated protein kinase kinase 1/2 (MEK1/2) and extracellular-signal–regulated kinases 1/2 (ERK 1/2) pathways and its binding to the myocardin promoter, leading to an increased expression of myocardin. The overall effect favors smooth muscle gene expression and cell differentiation toward smooth muscle cells.

Human iPS cells derived from patients with type 1 diabetes mellitus display a similar differentiation efficiency when compared with iPS cells derived from healthy donors.[76] These patient-derived iPS cells can differentiate into endothelial lineages expressing typical endothelial markers and with functional capabilities of lectin binding, acetylated low-density lipoprotein uptake, tube formation in matrigel, and response to TNF-α. These endothelial cells could undergo morphogenesis and assemble into 3D networks when cultured in engineered hyaluronic acid hydrogels or in response to low oxygen in vivo and could incorporate into developing vasculature in zebrafish.[76] Similar results were also obtained in another study where human iPS cells derived from somatic tissue could differentiate into both endothelial cells and pericytes which were fully functional both in vivo and in vitro.[77]

In investigation into the mechanisms of iPS cell differentiation, Chen et al[78] found that miR-199b is involved in endothelial differentiation. A step-wise increase in expression of miR-199 was detected during endothelial differentiation. Notably, miR-199b targeted the Notch ligand jagged1 (JAG1), resulting in VEGF transcriptional activation and secretion through the transcription factor signal transducer and activator of transcription 3 (STAT3). On shRNA-mediated knockdown of the Notch ligand JAG1, the regulatory effect of miR-199b was ablated,

and there was robust induction of STAT3 and VEGF during endothelial differentiation.[78] Using an in vitro tube formation assay and implanted Matrigel plugs in vivo, miR-199b was also shown to regulate VEGF expression and angiogenesis. miR-199b can thus be said to play a novel role as a regulator of the phenotypic switch during vascular cell differentiation from iPS cells by regulating critical angiogenic signaling responses.

Direct Reprogramming of Vascular Cells

Transplantation of endothelial cells is a promising treatment for the repair of damaged blood vessels and the growth of new ones after ischemia. However, in development of strategies based on the differentiation of pluripotent stem cells, researchers have found these cells to have limited proliferative potential and instable function, presenting a problem when trying to generate enough functional endothelial cells for therapy. To address this problem, new reprogramming protocols have recently been developed.[79] The transcription factor Ets variant 2 (Etv2) is known to be essential for the specification of endothelial and hematopoietic lineages in early gestation. Etv2 was found capable of directly converting primary human adult skin fibroblasts into functional endothelial cells in combination with forkhead box protein C2 (FOXC2) through a composite DNA-binding site.[79] In a more complex protocol, Etv2 was also shown to be able to directly reprogram human amniotic cells into endothelial cells without an intermediate pluripotent state. In this protocol, Etv2 is expressed for 2 weeks, followed by expression of the transcription factors friend leukemia integration 1 transcription factor (FLI1) and ETS-related gene 1 (ERG1), whereas TGF-β is inhibited for 3 weeks. Using this method, human mid-gestation lineage-committed amniotic fluid–derived cells were converted into a stable, functional, and proliferative population of vascular endothelial cells. Both of these protocols involving Etv2 could be promising in producing a large pool of endothelial cells for treatment.[80]

Direct reprogramming of adult cells can also be accomplished with the application of viral vectors encoding other transcription factors. In one study, overexpression of 11 candidate genes which are key regulators of endothelial development was achieved by lentiviral infection of adult skin fibroblasts from a Tie-GFP mouse, leading to an efficient reprogramming of skin fibroblasts into endothelial cells.[81] The function of derived endothelial cells was confirmed both in vitro and in vivo.[81] Sayed et al have recently pointed out that viral vectors are more than just passive vehicles for transcription factors because of their participation in the process of nuclear reprogramming to pluripotency. In a recent article, they demonstrated that toll-like receptor 3 agonist Poly I:C, along with basic fibroblast growth factor, VEGF, and bone morphogenetic protein-4, can transdifferentiate human fibroblasts into endothelial cells. These induced endothelial cells were also verified by a series of functional assays both in vivo and in vitro.[82]

Summary

Stem/progenitor cells present a promising future treatment for regeneration of the vasculature. Though the existence of both circulating and resident vascular progenitor cells, capable of differentiation into both endothelial and smooth muscle cells,

has been widely accepted over the last decade, there is, as yet, no gold standard surface marker profile or isolation procedure for these types of cells.[12] An alternative potential cell source for vascular regeneration is mesenchymal stem cells, and many new cell types, such as adipose–derived stem cells, have recently been discovered. Generating a large quantity of functional endothelial cells will be a key step in the treatment of vascular ischemic disorders. A series of studies into stem cells and induced pluripotent stem cells have presented impressive advances in our understanding of both the mechanisms behind cell differentiation into endothelial cells and animal models of transplantation of stem cell–derived endothelial cells after vascular injury. However, there are few clinical reports addressing the potential benefits and complications of stem cell transplantation in patients with ischemic disorders. Long-term safety data will also be required before any clinical application. Although there are still many obstacles to overcome, we hope that clinical application of stem cell therapy can be widely performed in the near future.

Disclosures

None.

References

1. Zhang B, Pu WT. Notching up vascular regeneration. *Cell Res*. 2014;24:777–778. doi: 10.1038/cr.2014.68.
2. Leeper NJ, Hunter AL, Cooke JP. Stem cell therapy for vascular regeneration: adult, embryonic, and induced pluripotent stem cells. *Circulation*. 2010;122:517–526. doi: 10.1161/CIRCULATIONAHA.109.881441.
3. Sun G, Gerecht S. Vascular regeneration: engineering the stem cell microenvironment. *Regen Med*. 2009;4:435–447. doi: 10.2217/rme.09.1.
4. Ma Q, Xia X, Tao Q, Lu K, Shen J, Xu Q, Hu X, Tang Y, Block NL, Webster KA, Schally AV, Wang J, Yu H. Profound actions of an agonist of growth hormone-releasing hormone on angiogenic therapy by mesenchymal stem cells. *Arterioscler Thromb Vasc Biol*. 2016. doi: 10.1161/ATVBAHA.116.307126.
5. Gupta RM, Meissner TB, Cowan CA, Musunuru K. Genome-edited human pluripotent stem cell-derived macrophages as a model of reverse cholesterol transport-brief report. *Arterioscler Thromb Vasc Biol*. 2016;36:15–18. doi: 10.1161/ATVBAHA.115.305956.
6. Kim JY, Choi JS, Song SH, Im JE, Kim JM, Kim K, Kwon S, Shin HK, Joo CK, Lee BH, Suh W. Stem cell factor is a potent endothelial permeability factor. *Arterioscler Thromb Vasc Biol*. 2014;34:1459–1467. doi: 10.1161/ATVBAHA.114.303575.
7. 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*. 1997;275:964–967.
8. 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*. 2001;89:E1–E7.
9. Chavakis E, Dernbach E, Hermann C, Mondorf UF, Zeiher AM, Dimmeler S. Oxidized LDL inhibits vascular endothelial growth factor-induced endothelial cell migration by an inhibitory effect on the Akt/endothelial nitric oxide synthase pathway. *Circulation*. 2001;103:2102–2107.

10. Dimmeler S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, Rütten H, Fichtlscherer S, Martin H, Zeiher AM. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J Clin Invest*. 2001;108:391–397. doi: 10.1172/JCI13152.

11. DeJarnett N, Yeager R, Conklin DJ, et al. Residential proximity to major roadways is associated with increased levels of AC133+ circulating angiogenic cells. *Arterioscler Thromb Vasc Biol*. 2015;35:2468–2477. doi: 10.1161/ATVBAHA.115.305724.

12. Hirschi KK, Ingram DA, Yoder MC. Assessing identity, phenotype, and fate of endothelial progenitor cells. *Arterioscler Thromb Vasc Biol*. 2008;28:1584–1595. doi: 10.1161/ATVBAHA.107.155960.

13. Pula G, Mayr U, Evans C, Prokopi M, Vara DS, 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 cell cultures. *Circ Res*. 2009;104:32–40. doi: 10.1161/CIRCRESAHA.108.182261.

14. Zampetaki A, Kirton JP, Xu Q. Vascular repair by endothelial progenitor cells. *Cardiovasc Res*. 2008;78:413–421. doi: 10.1093/cvr/cvn081.

15. Romagnani P, Annunziato F, Liotta F, et al. CD14+CD34low cells with stem cell phenotypic and functional features are the major source of circulating endothelial progenitors. *Circ Res*. 2005;97:314–322. doi: 10.1161/01.RES.0000177670.72216.9b.

16. Sharpe EE III, Teleron AA, Li B, Price J, Sands MS, Alford K, Young PP. The origin and *in vivo* significance of murine and human culture-expanded endothelial progenitor cells. *Am J Pathol*. 2006;168:1710–1721. doi: 10.2353/ajpath.2006.050556.

17. Prokopi M, Pula G, Mayr U, Devue C, Gallagher J, Xiao Q, Boulanger CM, Westwood N, Urbich C, Willeit J, Steiner M, Breuss J, Xu Q, Kiechl S, Mayr M. Proteomic analysis reveals presence of platelet microparticles in endothelial progenitor cell cultures. *Blood*. 2009;114:723–732. doi: 10.1182/blood-2009-02-205930.

18. Kirton JP, Xu Q. Endothelial precursors in vascular repair. *Microvasc Res*. 2010;79:193–199. doi: 10.1016/j.mvr.2010.02.009.

19. Yiu KH, Tse HF. Specific role of impaired glucose metabolism and diabetes mellitus in endothelial progenitor cell characteristics and function. *Arterioscler Thromb Vasc Biol*. 2014;34:1136–1143. doi: 10.1161/ATVBAHA.114.302192.

20. Yuldasheva NY, Rashid ST, Haywood NJ, et al. Haploinsufficiency of the insulin-like growth factor-1 receptor enhances endothelial repair and favorably modifies angiogenic progenitor cell phenotype. *Arterioscler Thromb Vasc Biol*. 2014;34:2051–2058. doi: 10.1161/ATVBAHA.114.304121.

21. Lee JH, Lee SH, Yoo SY, Asahara T, Kwon SM. CD34 hybrid cells promote endothelial colony-forming cell bioactivity and therapeutic potential for ischemic diseases. *Arterioscler Thromb Vasc Biol*. 2013;33:1622–1634. doi: 10.1161/ATVBAHA.112.301052.

22. Diao Y, Mohandas R, Lee P, Liu Z, Sautina L, Mu W, Li S, Wen X, Croker B, Segal MS. Effects of Long-Term Type I Interferon on the Arterial Wall and Smooth Muscle Progenitor Cells Differentiation. *Arterioscler Thromb Vasc Biol*. 2016;36:266–273. doi: 10.1161/ATVBAHA.115.306767.

23. Kim J, Kim M, Jeong Y, Lee WB, Park H, Kwon JY, Kim YM, Hwang D, Kwon YG. BMP9 Induces Cord Blood-Derived Endothelial Progenitor Cell Differentiation and Ischemic Neovascularization via ALK1. *Arterioscler Thromb Vasc Biol*. 2015;35:2020–2031. doi: 10.1161/ATVBAHA.115.306142.

24. Zhang D, Sun X, Liu J, Xie X, Cui W, Zhu Y. Homocysteine accelerates senescence of endothelial cells via DNA hypomethylation of human telomerase reverse transcriptase. *Arterioscler Thromb Vasc Biol*. 2015;35:71–78. doi: 10.1161/ATVBAHA.114.303899.

25. Lakshmipathy U, Davila J, Hart RP. miRNA in pluripotent stem cells. *Regen Med*. 2010;5:545–555. doi: 10.2217/rme.10.34.

26. Wang JM, Tao J, Chen DD, Cai JJ, Irani K, Wang Q, Yuan H, Chen AF. MicroRNA miR-27b rescues bone marrow-derived angiogenic cell function and accelerates wound healing in type 2 diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 2014;34:99–109. doi: 10.1161/ATVBAHA.113.302104.

27. Wang HW, Huang TS, Lo HH, Huang PH, Lin CC, Chang SJ, Liao KH, Tsai CH, Chan CH, Tsai CF, Cheng YC, Chiu YL, Tsai TN, Cheng CC, Cheng SM. Deficiency of the microRNA-31-microRNA-720 pathway in the plasma and endothelial progenitor cells from patients with coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2014;34:857–869. doi: 10.1161/ATVBAHA.113.303001.

28. Zhao H, Wen G, Wen G, Huang Y, Yu X, Chen Q, Afzal TA, Luong le A, Zhu J, Ye S, Shu Y, Zhang L, Xiao Q. MicroRNA-22 regulates smooth muscle cell differentiation from stem cells by targeting methyl CpG-binding protein 2. *Arterioscler Thromb Vasc Biol*. 2015;35:918–929. doi: 10.1161/ATVBAHA.114.305212.

29. Zernecke A, Weber C. Chemokines in atherosclerosis: proceedings resumed. *Arterioscler Thromb Vasc Biol*. 2014;34:742–750. doi: 10.1161/ATVBAHA.113.301655.

30. Hiasa K, Ishibashi M, Ohtani K, Inoue S, Zhao Q, Kitamoto S, Sata M, Ichiki T, Takeshita A, Egashira K. Gene transfer of stromal cell-derived factor-1alpha enhances ischemic vasculogenesis and angiogenesis via vascular endothelial growth factor/endothelial nitric oxide synthase-related pathway: next-generation chemokine therapy for therapeutic neovascularization. *Circulation*. 2004;109:2454–2461. doi: 10.1161/01.CIR.0000128213.96779.61.

31. Friedrich EB, Walenta K, Scharlau J, Nickenig G, Werner N. CD34-/CD133+/VEGFR-2+ endothelial progenitor cell subpopulation with potent vasoregenerative capacities. *Circ Res*. 2006;98:e20–e25. doi: 10.1161/01.RES.0000205765.28940.93.

32. Kobayashi K, Sato K, Kida T, Omori K, Hori M, Ozaki H, Murata T. Stromal cell-derived factor-1α/C-X-C chemokine receptor type 4 axis promotes endothelial cell barrier integrity via phosphoinositide 3-kinase and Rac1 activation. *Arterioscler Thromb Vasc Biol*. 2014;34:1716–1722. doi: 10.1161/ATVBAHA.114.303890.

33. Noels H, Zhou B, Tilstam PV, et al. Deficiency of endothelial CXCR4 reduces reendothelialization and enhances neointimal hyperplasia after vascular injury in atherosclerosis-prone mice. *Arterioscler Thromb Vasc Biol*. 2014;34:1209–1220. doi: 10.1161/ATVBAHA.113.302878.

34. Subramanian S, Liu C, Aviv A, Ho JE, Courchesne P, Muntendam P, Larson MG, Cheng S, Wang TJ, Mehta NN, Levy D. Stromal cell-derived factor 1 as a biomarker of heart failure and mortality risk. *Arterioscler Thromb Vasc Biol*. 2014;34:2100–2105. doi: 10.1161/ATVBAHA.114.303579.

35. Kiemer AK, Weber NC, Fürst R, Bildner N, Kulhanek-Heinze S, Vollmar AM. Inhibition of p38 MAPK activation via induction of MKP-1: atrial natriuretic peptide reduces TNF-alpha-induced actin polymerization and endothelial permeability. *Circ Res*. 2002;90:874–881.

36. Wong MM, Chen Y, Margariti A, Winkler B, Campagnolo P, Potter C, Hu Y, Xu Q. Macrophages control vascular stem/progenitor cell plasticity through tumor necrosis factor-α-mediated nuclear factor-κB activation. *Arterioscler Thromb Vasc Biol*. 2014;34:635–643. doi: 10.1161/ATVBAHA.113.302568.

37. Zhang L, Xu Q. Stem/Progenitor cells in vascular regeneration. *Arterioscler Thromb Vasc Biol*. 2014;34:1114–1119. doi: 10.1161/ATVBAHA.114.303809.

38. Hu Y, Zhang Z, Torsney E, Afzal AR, Davison F, Metzler B, Xu Q. Abundant progenitor cells in the adventitia contribute to atherosclerosis of vein grafts in ApoE-deficient mice. *J Clin Invest*. 2004;113:1258–1265. doi: 10.1172/JCI19628.

39. Sainz J, Al Haj Zen A, Caligiuri G, Demerens C, Urbain D, Lemitre M, Lafont A. Isolation of "side population" progenitor cells from healthy arteries of adult mice. *Arterioscler Thromb Vasc Biol*. 2006;26:281–286. doi: 10.1161/01.ATV.0000197793.83391.91.

40. Ingram DA, Mead LE, Moore DB, Woodard W, Fenoglio A, Yoder MC. Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells. *Blood*. 2005;105:2783–2786. doi: 10.1182/blood-2004-08-3057.

41. Covas DT, Piccinato CE, Orellana MD, Siufi JL, Silva WA Jr, Proto-Siqueira R, Rizzatti EG, Neder L, Silva AR, Rocha V, Zago MA. Mesenchymal stem cells can be obtained from the human saphena vein. *Exp Cell Res*. 2005;309:340–344. doi: 10.1016/j.yexcr.2005.06.005.

42. Torsney E, Xu Q. Resident vascular progenitor cells. *J Mol Cell Cardiol*. 2011;50:304–311. doi: 10.1016/j.yjmcc.2010.09.006.

43. Psaltis PJ, Puranik AS, Spoon DB, Chue CD, Hoffman SJ, Witt TA, Delacroix S, Kleppe LS, Mueske CS, Pan S, Gulati R, Simari RD. Characterization of a resident population of adventitial macrophage progenitor cells in postnatal vasculature. *Circ Res*. 2014;115:364–375. doi: 10.1161/CIRCRESAHA.115.303299.

44. Iacobazzi D, Mangialardi G, Gubernator M, Hofner M, Wielscher M, Vierlinger K, Reni C, Oikawa A, Spinetti G, Vono R, Sangalli E, Montagnani M, Madeddu P. Increased antioxidant defense mechanism in human adventitia-derived progenitor cells is associated with therapeutic benefit in ischemia. *Antioxid Redox Signal*. 2014;21:1591–1604. doi: 10.1089/ars.2013.5404.

45. Gubernator M, Slater SC, Spencer HL, et al. Epigenetic profile of human adventitial progenitor cells correlates with therapeutic outcomes in a mouse model of limb ischemia. *Arterioscler Thromb Vasc Biol*. 2015;35:675–688. doi: 10.1161/ATVBAHA.114.304989.

46. Campagnolo P, Hong X, di Bernardini E, Smyrnias I, Hu Y, Xu Q. Resveratrol-Induced Vascular Progenitor Differentiation towards Endothelial Lineage via MiR-21/Akt/β-Catenin Is Protective in Vessel Graft Models. *PLoS One*. 2015;10:e0125122. doi: 10.1371/journal.pone.0125122.

47. Frid MG, Brunetti JA, Burke DL, Carpenter TC, Davie NJ, Reeves JT, Roedersheimer MT, van Rooijen N, Stenmark KR. Hypoxia-induced pulmonary vascular remodeling requires recruitment of circulating mesenchymal precursors of a monocyte/macrophage lineage. *Am J Pathol*. 2006;168:659–669. doi: 10.2353/ajpath.2006.050599.

48. Nadaud S, Dierick F, Tiphaine H, Monceau V, Mougenot N, Crisan M, Dorfmuller P, Marodon G, Besson V, Marazzi G. Lung progenitor cells expressing pw1 gene participate in vascular remodeling during pulmonary arterial hypertension. *FASEB J*. 2015;29:LB569.

49. Mao SZ, Ye X, Liu G, Song D, Liu SF. Resident Endothelial Cells and Endothelial Progenitor Cells Restore Endothelial Barrier Function After Inflammatory Lung Injury. *Arterioscler Thromb Vasc Biol*. 2015;35:1635–1644. doi: 10.1161/ATVBAHA.115.305519.

50. Zaniboni A, Bernardini C, Alessandri M, Mangano C, Zannoni A, Bianchi F, Sarli G, Calzà L, Bacci ML, Forni M. Cells derived from porcine aorta tunica media show mesenchymal stromal-like cell properties in *in vitro* culture. *Am J Physiol Cell Physiol*. 2014;306:C322–C333. doi: 10.1152/ajpcell.00112.2013.

51. Zhang C, Zeng L, Emanueli C, Xu Q. Blood flow and stem cells in vascular disease. *Cardiovasc Res*. 2013;99:251–259. doi: 10.1093/cvr/cvt061.

52. Potter CM, Lao KH, Zeng L, Xu Q. Role of biomechanical forces in stem cell vascular lineage differentiation. *Arterioscler Thromb Vasc Biol*. 2014;34:2184–2190. doi: 10.1161/ATVBAHA.114.303423.

53. Abedin M, Tintut Y, Demer LL. Mesenchymal stem cells and the artery wall. *Circ Res*. 2004;95:671–676. doi: 10.1161/01.RES.0000143421.27684.12.

54. Ding DC, Shyu WC, Lin SZ. Mesenchymal stem cells. *Cell Transplant*. 2011;20:5–14. doi: 10.3727/096368910X.

55. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284:143–147.

56. Zhu H, Sun A, Zhu H, Li Z, Huang Z, Zhang S, Ma X, Zou Y, Hu K, Ge J. Aldehyde dehydrogenase-2 is a host factor required for effective bone marrow mesenchymal stem cell therapy. *Arterioscler Thromb Vasc Biol*. 2014;34:894–901. doi: 10.1161/ATVBAHA.114.303241.

57. Zhu H, Sun A, Zou Y, Ge J. Inducible metabolic adaptation promotes mesenchymal stem cell therapy for ischemia: A hypoxia-induced and glycogen-based energy prestorage strategy. *Arterioscler Thromb Vasc Biol*. 2014;34:870–876. doi: 10.1161/ATVBAHA.114.303194.

58. Liu X, Sun X, Liao H, et al. Mitochondrial aldehyde dehydrogenase 2 regulates revascularization in chronic ischemia: potential impact on the development of coronary collateral circulation. *Arterioscler Thromb Vasc Biol*. 2015;35:2196–2206. doi: 10.1161/ATVBAHA.115.306012.

59. Crisan M, Corselli M, Chen WC, Péault B. Perivascular cells for regenerative medicine. *J Cell Mol Med*. 2012;16:2851–2860. doi: 10.1111/j.1582-4934.2012.01617.x.

60. Preda MB, Rønningen T, Burlacu A, Simionescu M, Moskaug JØ, Valen G. Remote transplantation of mesenchymal stem cells protects the heart against ischemia-reperfusion injury. *Stem Cells*. 2014;32:2123–2134. doi: 10.1002/stem.1687.

61. Olea FD, Locatelli P, Hnatiuk A, De Lorenzi A, Valdivieso L, Rocha E, Ramírez R, Laguens R, Crottogini A. Vascular endothelial growth factor overexpression does not enhance adipose stromal cell-induced protection on muscle damage in critical limb ischemia. *Arterioscler Thromb Vasc Biol*. 2015;35:184–188. doi: 10.1161/ATVBAHA.114.304348.

62. Katare R, Stroemer P, Hicks C, Stevanato L, Patel S, Corteling R, Miljan E, Vishnubhatla I, Sinden J, Madeddu P. Clinical-grade human neural stem cells promote reparative neovascularization in mouse models of hindlimb ischemia. *Arterioscler Thromb Vasc Biol*. 2014;34:408–418. doi: 10.1161/ATVBAHA.113.302592.

63. Wirrig EE, Yutzey KE. Conserved transcriptional regulatory mechanisms in aortic valve development and disease. *Arterioscler Thromb Vasc Biol*. 2014;34:737–741. doi: 10.1161/ATVBAHA.113.302071.

64. Mathur A, Martin JF. Stem cells and repair of the heart. *Lancet*. 2004;364:183–192. doi: 10.1016/S0140-6736(04)16632-4.

65. Wang M, Subramanian M, Abramowicz S, Murphy AJ, Gonen A, Witztum J, Welch C, Tabas I, Westerterp M, Tall AR. Interleukin-3/ granulocyte macrophage colony-stimulating factor receptor promotes stem cell expansion, monocytosis, and atheroma macrophage burden in mice with hematopoietic ApoE deficiency. *Arterioscler Thromb Vasc Biol*. 2014;34:976–984. doi: 10.1161/ATVBAHA.113.303097.

66. Gao M, Zhao D, Schouteden S, Sorci-Thomas MG, Van Veldhoven PP, Eggermont K, Liu G, Verfaillie CM, Feng Y. Regulation of high-density lipoprotein on hematopoietic stem/progenitor cells in atherosclerosis requires scavenger receptor type BI expression. *Arterioscler Thromb Vasc Biol*. 2014;34:1900–1909. doi: 10.1161/ATVBAHA.114.304006.

67. Murphy AJ, Sarrazy V, Wang N, Bijl N, Abramowicz S, Westerterp M, Welch CB, Schuetz JD, Yvan-Charvet L. Deficiency of ATP-binding cassette transporter B6 in megakaryocyte progenitors accelerates atherosclerosis in mice. *Arterioscler Thromb Vasc Biol*. 2014;34:751–758. doi: 10.1161/ATVBAHA.113.302613.

68. Rippon HJ, Bishop AE. Embryonic stem cells. *Cell Prolif*. 2004;37:23–34.

69. Luo Z, Wang G, Wang W, Xiao Q, Xu Q. Signalling pathways that regulate endothelial differentiation from stem cells. *Front Biosci (Landmark Ed)*. 2011;16:472–485.

70. Masuda S, Matsuura K, Anazawa M, Iwamiya T, Shimizu T, Okano T. Formation of vascular network structures within cardiac cell sheets from mouse embryonic stem cells. *Regenerative Therapy*. 2015;2:6–16.

71. Wu L, Wary KK, Revskoy S, Gao X, Tsang K, Komarova YA, Rehman J, Malik AB. Histone Demethylases KDM4A and KDM4C Regulate Differentiation of Embryonic Stem Cells to Endothelial Cells. *Stem Cell Reports*. 2015;5:10–21. doi: 10.1016/j.stemcr.2015.05.016.

72. Xiao Q, Wang G, Luo Z, Xu Q. The mechanism of stem cell differentiation into smooth muscle cells. *Thromb Haemost*. 2010;104:440–448. doi: 10.1160/TH09-11-0794.

73. Wang X, Karamariti E, Simpson R, Wang W, Xu Q. Dickkopf Homolog 3 Induces Stem Cell Differentiation into Smooth Muscle Lineage via ATF6 Signalling. *J Biol Chem*. 2015;290:19844–19852. doi: 10.1074/jbc.M115.641415.

74. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663–676. doi: 10.1016/j.cell.2006.07.024.

75. Kusuma S, Peijnenburg E, Patel P, Gerecht S. Low oxygen tension enhances endothelial fate of human pluripotent stem cells. *Arterioscler Thromb Vasc Biol*. 2014;34:913–920. doi: 10.1161/ATVBAHA.114.303274.

76. Chan XY, Black R, Dickerman K, Federico J, Lévesque M, Mumm J, Gerecht S. Three-Dimensional Vascular Network Assembly From Diabetic Patient-Derived Induced Pluripotent Stem Cells. *Arterioscler Thromb Vasc Biol*. 2015;35:2677–2685. doi: 10.1161/ATVBAHA.115.306362.

77. Orlova VV, Drabsch Y, Freund C, Petrus-Reurer S, van den Hil FE, Muenthaisong S, Dijke PT, Mummery CL. Functionality of endothelial cells and pericytes from human pluripotent stem cells demonstrated in cultured vascular plexus and zebrafish xenografts. *Arterioscler Thromb Vasc Biol*. 2014;34:177–186. doi: 10.1161/ATVBAHA.113.302598.

78. Chen T, Margariti A, Kelaini S, Cochrane A, Guha ST, Hu Y, Stitt AW, Zhang L, Xu Q. MicroRNA-199b Modulates Vascular Cell Fate During iPS Cell Differentiation by Targeting the Notch Ligand Jagged1 and Enhancing VEGF Signaling. *Stem Cells*. 2015;33:1405–1418. doi: 10.1002/stem.1930.

79. Morita R, Suzuki M, Kasahara H, Shimizu N, Shichita T, Sekiya T, Kimura A, Sasaki K, Yasukawa H, Yoshimura A. ETS transcription factor ETV2 directly converts human fibroblasts into functional endothelial cells. *Proc Natl Acad Sci U S A*. 2015;112:160–165. doi: 10.1073/pnas.1413234112.

80. Ginsberg M, Schachterle W, Shido K, Rafii S. Direct conversion of human amniotic cells into endothelial cells without transitioning through a pluripotent state. *Nat Protoc*. 2015;10:1975–1985. doi: 10.1038/nprot.2015.126.

81. Han JK, Chang SH, Cho HJ, Choi SB, Ahn HS, Lee J, Jeong H, Youn SW, Lee HJ, Kwon YW, Cho HJ, Oh BH, Oettgen P, Park YB, Kim HS. Direct conversion of adult skin fibroblasts to endothelial cells by defined factors. *Circulation*. 2014;130:1168–1178. doi: 10.1161/CIRCULATIONAHA.113.007727.

82. Sayed N, Wong WT, Ospino F, Meng S, Lee J, Jha A, Dexheimer P, Aronow BJ, Cooke JP. Transdifferentiation of human fibroblasts to endothelial cells: role of innate immunity. *Circulation*. 2015;131:300–309. doi: 10.1161/CIRCULATIONAHA.113.007394.

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American
Heart
Association®

Vascular Regeneration by Stem/Progenitor Cells
Yao Xie, Ye Fan and Qingbo Xu

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/36/5/e33