Diabetes Mellitus and Ischemic Diseases
Molecular Mechanisms of Vascular Repair Dysfunction

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Objective—In patients with diabetes mellitus, the ability of ischemic tissue to synchronize the molecular and cellular events leading to restoration of tissue perfusion in response to the atherosclerotic occlusion of a patent artery is markedly impaired. As a consequence, adverse tissue remodeling and the extent of ischemic injury are intensified, leading to increased morbidity and mortality.

Approach and Results—Growing evidence from preclinical and clinical studies has implicated alterations in hypoxia-inducible factor 1 levels in the abrogation of proangiogenic pathways, including vascular endothelial growth factor A/phosphoinositide 3′ kinase/AKT/endothelial nitric oxide synthase and in the activation of antiangiogenic signals characterized by accumulation of advanced glycation end products, reactive oxygen species overproduction, and endoplasmic reticulum stress. In addition, the diabetic milieu shows a switch toward proinflammatory antiregenerative pathways. Finally, the mobilization, subsequent recruitment, and the proangiogenic potential of the different subsets of angiogenesis-promoting bone marrow–derived cells are markedly impaired in the diabetic environment.

Conclusions—In this review, we will give an overview of the current understanding on the signaling molecules contributing to the diabetes mellitus–induced impairment of posts ischemic revascularization mainly in the setting of myocardial infarction or critical limb ischemia. (Arterioscler Thromb Vasc Biol. 2014;34:00-00.)

Key Words: angiogenesis effect • anoxia • diabetes mellitus • inflammation • intercellular signaling peptides and proteins • ischemia • stem cells

In patients with type 1 (T1D) and type 2 diabetes mellitus (T2D), cardiovascular disease is the most common cause of death, 45% and 52%, respectively. In particular, diabetes mellitus is associated with micro- and macrovascular complications resulting in coronary heart disease and increased morbidity and mortality. Similarly, patients with diabetes mellitus are at high risk for peripheral arterial disease characterized by symptoms of intermittent claudication or critical limb ischemia (CLI). 1 These vascular complications include both qualitative and quantitative adjustments of the vascular architecture. In particular, the negative outcome of patient with diabetes mellitus is partly related to the abrogation of new vessel formation and remodeling of the pre-existing vasculature that represent an integral component of tissue remodeling and control the extent of ischemic injury. In addition, vascular dysfunction, mainly characterized by alteration of the mechanical properties of the arterial wall and functional disruption of the endothelium, is associated with diabetes mellitus and likely fuel the pathogenesis of vascular disease in T1D and T2D. Therefore, the present review focuses on the molecular mechanisms involved in the vascular defects associated with diabetes mellitus–induced posts ischemic vessel growth in the setting of myocardial infarction (MI) or CLI.

Molecular Mechanisms of Diabetes Mellitus–Induced Dysfunction of the Angiogenic Competence

Hypoxia-Inducible Factor 1

Hypoxia, at least in part through activation of the hypoxia-inducible factor 1 (HIF-1) α-related pathways, controls all steps of the posts ischemic revascularization process. Recent studies uncover that destabilization of HIF-1 is most likely the event that transduces hyperglycemia into the loss of the cellular response to hypoxia in most diabetic complications. 2 Levels of HIF-1α are decreased in biopsies from foot ulcers of patients with diabetes mellitus as compared with venous ulcers that share the same hypoxic environment but are not exposed to hyperglycemia. 3 Downregulation of HIF-1 in response to hyperglycemia also seems to account for the decreased collateral growth triggered by myocardial ischemia in patients with diabetes mellitus. 4 A human HIF-1α genetic polymorphism is related with both type 2 diabetes mellitus 5 and the absence of coronary collaterals in patients with ischemic heart disease. 6 In an experimental model of MI in rats, infarct size increases in response to hyperglycemia in association with reduced

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production of the HIF-1α protein. In diabetic db/db mice, the intramuscular administration of an adenovirus encoding a constitutively active form of HIF-1α enhances tissue perfusion and vascular remodeling in animals with CLI. High glucose has been shown to decrease the associations between both HIF-1α/HIF-1β and HIF-1α/p300. The iron chelator, DFO, improves HIF-1α-p300 binding and augments HIF-1 activity at high glucose levels, by preventing p300 modification by the advanced glycation end products (AGEs), methylglyoxal, through the decreased production of reactive oxygen species (ROS). Other studies have suggested a role for the prolylhydroxylase domain proteins (PHDs) in impairment of the HIF-mediated cellular response to hypoxia during hyperglycemia. Indeed, inhibition of hydroxylases by dimethyloxalylglycine, an oxoglutarate analogue known to be a potent inhibitor of PHDs, or by the iron chelator DFO, prevents the repressive effects of high glucose levels on HIF-1α protein stability and activity, as well as on the expression of HIF-1 target genes.

In this line, the use of specific siRNAs directed against the 3 forms of PHDs induces the postischemic revascularization process in both diabetic and non-diabetic mice (Figure).

**Vascular Endothelial Growth Factor–Related Pathways**

As a consequence of the blunted cellular response to hypoxia, most of the HIF-1α-targeted genes are reduced in the setting of diabetes mellitus. Hence, vascular endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR2) contents are downregulated in ventricles from patients with diabetes mellitus. Similarly, mRNA and protein levels of VEGF-A and its receptors VEGFR1 and VEGFR2 are decreased in diabetic rats. The reduction in neovascularization in the non-obese diabetic mice is also associated with a lower level of VEGF-A, and normal levels of neovascularization could be achieved in non-obese diabetic mice that have been treated with intramuscular injection of an adenoviral vector encoding for VEGF. Soluble VEGFR1, an angiogenesis inhibitor, is also upregulated in skeletal muscle by T2D and ischemia and likely reduces the capacity of VEGF to induce an angiogenic response in the setting of diabetes mellitus. VEGF-A may activate cell proliferation and survival, through mechanisms dependent on phosphatidylinositol 3′ kinase/AKT/endothelial nitric oxide synthase (eNOS). In a model of peripheral ischemia in mice, the angiogenic response to VEGF-A is dependent on eNOS administration by gene therapy promotes neovascularization in the ischemic legs of control and diabetic animals, highlighting the importance of NO in this context. The levels of biologically active NO are also dependent on the production of ROS in the diabetic milieu. ROS, such as the superoxide anion, hydrogen peroxide and the hydroxyl radical, and nitrogen derivatives, such as peroxynitrite, are biologically active radicals that activate multiple signaling pathways through their oxidoreductive potential. The excessive accumulation of ROS is associated with defective postischemic revascularization in diabetic or hypercholesterolemic mice. In addition, administration of an adenoviral vector encoding the antioxidant thioredoxin-1 increases capillary and arteriolar density and restores cardiac function in diabetic infarcted heart.
therapy completely restores ischemic hindlimb blood flow in mice with T2D. Nitrite therapy significantly increases ischemic tissue VEGF protein expression that is essential for nitrite-mediated reperfusion of ischemic hind limbs. Nitrite also enhances ischemic tissue NO bioavailability along with concomitant reduction of superoxide formation. ROS can also trigger endoplasmic reticulum stress that has emerged as a major site of cellular homeostasis. In particular, C/EBP homologous protein-10 has been identified as an endoplasmic reticulum stress–induced transcription factor that can inhibit eNOS gene transcription and subsequently postischemic revascularization. In this line, induction of diabetes mellitus is associated with a marked upregulation of C/EBP homologous protein-10 that substantially hampers vessel growth in the setting of ischemia.

### Additional Angiogenic-Related Pathways

The expression patterns of other numerous angiogenesis-related proteins are also altered in diabetic tissue after ischemic injury. These transcripts include neuropilin-1 (Nrp1), placental growth factor, elastin, and matrix metalloproteinases implicated in blood vessel growth and maintenance of vessel wall integrity. In contrast, the antiangiogenic proteins, angiostatin and endostatin, are significantly elevated in the diabetic myocardium. In addition, high glucose treatment of endothelial cells results in a significant decrease in hepatocyte growth factor levels and a subsequent inhibition of matrix metalloproteinase-1 protein and eNOS expression in human aortic endothelial cells. Intramuscular injection of human hepatocyte growth factor plasmid induces therapeutic angiogenesis in a rat diabetic hindlimb ischemia model. Downregulation of PDGF-CC expression in limb tissues of diabetic mice has been shown to contribute to impaired angiogenesis in the diabetic state. The expression of PDGF-BB is also impaired in the TID mice on baseline, as well as over a time course after limb ischemia. Inhibition of overproduction of AGEs elicits dephosphorylation of PKC and restores expression of PDGF-BB irrespective of blood sugar levels, indicating that AGE is an essential regulator for PKC/PDGFB-B in the setting of diabetes mellitus. AGEs also likely control multiple steps of postischemic revascularization. Indeed, overexpression of GLO1, which encodes glyoxalase 1, the rate-limiting enzyme in the detoxification of AGEs, methylglyoxal, prevents the reduction of VEGF levels observed in those conditions. Treatment with angiotatin reduces AGE plasma levels and completely normalizes ischemia-induced revascularization in diabetic mice. This effect is probably mediated by restoration of matrix degradation processes that are disturbed as a result of AGE accumulation. Alternatively, the diabetes mellitus–induced decrease in VEGF and PDGF mRNA levels is prevented in diabetic mice lacking the PKCh isoform. As a consequence, PKCh-deficient diabetic mice show significantly increased blood flow, capillary density, and number of capillaries. Finally, angiopoietins and their receptor Tie-2 have been shown to harmonize vascular regeneration in diabetic tissue. Tie-2 expression is significantly reduced, whereas angiopoietin 2 (Ang-2) is increased in db/db mice subjected to MI. Overexpression of Ang-2 suppressed Tie-2 and VEGF expression in db/db mouse hearts together with significant upregulation of Wnt7b content. Overexpression of Ang-2 also sensitizes ICAM-1 and VCAM-1 expression in db/db mouse hearts. Interestingly, Ang-1–induced smooth muscle cells recruitment and vessel outgrowth are severely impaired in db/db mice. These alterations lead to a significant impairment of Ang-1–induced Akt and eNOS phosphorylation. Ang-1 gene transfer restores Tie-2 expression and rescues these abnormalities in diabetes mellitus. Ang-1 also increases myocardial vascular maturation and angiogenesis together with suppression of PHD2 and upregulation of HIF-1α signaling. Coadministration of VEGF and Ang-1 increases angiogenesis and reduces ventricular remodeling in the infarcted diabetic myocardium.

### Emerging Pathways

Numerous micro-RNAs have emerged as key regulator of hypoxia and growth factor–related pathways. miRNAs are clearly involved in postischemic revascularization and may participate to the diabetes mellitus–induced impairment in vessel growth and remodeling. Some miRNAs, such as miRNA 92a, miR-503, miRNA-15a, and miRNA-200c, are inhibitory, whereas others, such as miRNA-93, miRNA 126, and miRNA 210, activate postischemic revascularization processes in models of leg ischemia and MI. Hence, miR-503 expression is increased in ischemic limb muscles of T1D mice and in endothelial cells enriched from these muscles. Moreover, adenoviral administration of decoy miR-503 to the ischemic adductor of diabetic mice corrects diabetes mellitus–induced impairment of postischemic angiogenesis and blood flow recovery. Upregulation of miRNA-320 is also observed in myocardial microvascular endothelial cells from diabetic Goto-Kakizaki rats. The proliferation and migration of diabetic myocardial microvascular endothelial cell improve after transfection of the miR-320 inhibitor through increased expression of IGF-1 protein.

### Diabetes Mellitus and Inflammation-Related Pathways

#### Inflammation and Postischemic Revascularization

Distinct subsets of inflammatory cells are recruited to ischemic sites and affect the vascular and tissue remodeling in the context of leg ischemia or MI. In particular, the extent of the postischemic neovascularization process in animal model of CLI depends directly on the number of circulating monocytes. Consistent with these results, op/op mice lacking monocyte colony-stimulating factor and presenting monocytopenia display low levels of neovascularization. Monocytes constitute a heterogeneous population with 2 major subtypes in mice: Ly6ChiCCR2−CX3CR1low monocytes and Ly6C−CCR2−CX3CR1high monocytes corresponding to the CD14+CD16− and CD14+CD16+ subpopulations, respectively, in humans. The depletion of one of these subtypes of monocytes leads to changes in the process of angiogenesis in the myocardium. By contrast, in a leg ischemia model, the transplantation of Ly6Chi monocytes, but not of Ly6Clo monocytes, leads to activation of the processes of neovascularization. Two subtypes of macrophage can also be distinguished on the basis of various markers and
functionally different cell types. The M1 population expresses the inducible NOS and proinflammatory cytokines, such as interleukin-1 and interleukin-12, whereas the M2 population produces anti-inflammatory cytokines interleukin-10 and VEGF.

**Diabetes Mellitus and the Inflammatory Balance in Ischemic Milieu**

The diabetic macro- and microenvironment may alter the number as well as the activation and differentiation states of each cell type of inflammatory cells and subsequently negatively affects vessel growth. In this review, whereas the number of classical CD14\(^\text{Low}\)CD16\(^\text{−}\), intermediate CD14\(^\text{Intermediate}\)CD16\(^\text{+}\), and nonclassical CD14\(^\text{High}\)CD16\(^\text{−}\) monocyte is unchanged, T2D is characterized by an imbalanced M1/M2 ratio, attributable to a reduction in M2. The M1/M2 ratio is directly correlated with HbA1c, an indicator of glucose, and is associated with microangiopathy. Similarly, cardiac injury in Zucker diabetic fatty rats is associated with proinflammatory M1 phenotype and the heme oxygenase inducer, heme, reverses this defective phenotype through preferential polarization of macrophages toward anti-inflammatory macrophage M2. Diabetic mice have also increased numbers of circulating neutrophils and Ly6Chigh monocytes, reflecting hyperglycemia-induced proliferation and expansion of bone marrow (BM) myeloid progenitors and release of monocytes into the circulation. Increased neutrophil production of alarmin, S100A8/S100A9, and its subsequent interaction with the receptor for AGEs on common neutrophil production of alarmin, S100A8/S100A9, and its subsequent interaction with the receptor for AGEs on common neutrophil surface leads to enhanced myelopoiesis. Interestingly, treatment of hyperglycemia reduces monocyteosis in this setting. Finally, diabetic mice deficient for the modulating glycoprotein 130 receptor are protected from the development of diabetes mellitus–induced changes in the BM-derived cell mobilization.

Diabetes mellitus also likely affects the infiltration of inflammatory cells into the ischemic milieu. The cellular response of monocytes to VEGF-A is attenuated in patients with diabetes mellitus because of a downstream signal transduction defect. Mice lacking the chemokine receptor CCR2 or its ligand CCL2 display impaired revascularization associated with a low level of monocyte/macrophage infiltration in ischemic territories. The monocyte migration toward CCL2 is also strongly reduced in diabetic rabbits with CLI, leading to a reduced blood volume index in the region of growing collaterals. Baseline P-selectin level is 4-fold higher in T2D mice compared with wild-type mice but increases minimally at day 1 after CLI, whereas P-selectin content increases 10-fold in wild-type mice. Immunohistology of the hindlimb skeletal muscle demonstrates severely reduced monocyte recruitment in db/db mice compared with wild-type mice. Local treatment with CCL2 corrects the deficits in postischemic P-selectin expression and monocyte recruitment and leads to greater recovery in blood flow in db/db mice.

**Diabetes Mellitus and Angiogenesis-Promoting BM-Derived Cells**

During ischemia, the lesioned tissue recruits angiogenic cells, which are involved in the restoration of the vascular network. Numerous complex tissues contain reservoirs of stem/progenitor cells with vasculogenic potential as well as angiogenic cells with paracrine potential. In particular, the BM contained a large variety of these different subsets of cells. Acute ischemic diseases, such as MI and CLI, affect osteoblastic and vascular niches, inducing the transient mobilization of various types of cells (hematopoietic progenitor cells, endothelial progenitor cells [EPCs], and multiple proangiogenic cells) in humans and mice. Mobilization process is under the control of proteases, such as matrix metalloproteinases types 2 and 9, cathepsins, and the elastase produced by neutrophils. Important interactions occur with specific receptors, involving integrin α4 and VCAM-1. The mobilization of BM-derived cells also depends on gradients of chemokines, as described for CXCL12. Finally, mobilization of EPC from the BM also occurs after activation of peripheral noradrenergic neurons and release of norepinephrine, which suppresses osteoblast activity. This results in stromal eNOS activation, a local decrease in CXCL12 and subsequent EPC mobilization from the BM.

**Diabetes Mellitus and Angiogenic Cell Number**

T1D and T2D are associated with reduced EPC numbers. Activation of the Akt/p53/p21 signaling pathway and accelerated onset of senescence are detectable in EPC from patients with diabetes mellitus. Diabetic EPC depleted of endogenous p53 do not undergo senescence growth arrest and acquire the ability to form tube-like structures in vitro, identifying the activation of the p53 signaling pathway as a crucial event that can contribute to the impaired EPC number in diabetes mellitus. Interestingly, EPC decrease is related to peripheral arterial disease severity, strengthening the pathogenetic role of EPC dysregulation in diabetic vasculopathy.

**Diabetes Mellitus and BM-Derived Cell Mobilization**

Diabetes mellitus also activates apoptosis in BM-derived CD34\(^\text{+}\) cells through an upregulation and nuclear localization of the proapoptotic factor FOXO3a and induction of FOXO3a targets, p21 and p27(kip1). Moreover, microRNA-155, which regulates cell survival through inhibition of FOXO3a, is downregulated in diabetic BM-derived CD34\(^\text{+}\) cells and inversely correlated with FOXO3a levels. The decrease in the number of angiogenic cells may also be because of the diabetes mellitus–induced abrogation of BM-derived cell mobilization. Hence, in control rats, EPCs showed a mobilization curve within 7 days, whereas diabetic rats are completely unable to mobilize EPCs after ischemic injury. As a consequence, diabetic rats show no compensatory increase in muscle capillary density. Defective EPC mobilization in diabetes mellitus is associated with altered release of CXCL12 and VEGF and inability to upregulate muscle HIF-1α. Impaired blood perfusion in diabetic tissues is intimately associated with defective peripheral nerve function. Peripheral nerves are aligned with blood vessels and postganglionic sympathetic nerves contact arterial smooth muscle cells to control arterial tone. In T2D rats, the number of nerve terminal endings in the BM is reduced and such denervation is accompanied by a loss of circadian release of EPCs and a marked reduction in clock gene expression in diabetic tissue and in EPCs themselves. This reduction in the circadian peak of EPC release leads to diminished reparative
capacity. Of great interest, myocardial dysfunction, acute coronary syndrome, sudden cardiac death, and ischemic stroke occur with peak incidence in non-diabetic patients in the early morning, yet in patients with diabetes mellitus the peak is at night. Similarly, T1D and T2D or chemical sympathectomy in mice result in BM autonomic neuropathy, impaired Lin- cKit+Scal+ cell and EPC mobilization and vascular recovery after ischemia. This is associated with increased expression of p66Shc and reduced expression of Sirt1 in BM. Interestingly, proangiogenic growth factors, such as Desert Hedgehog, do not act on endothelial cells but promote postischemic revascularization through the maintenance of the pool of nerve-derived proangiogenic factors and the survival of peripheral nerve in the ischemic muscle. In addition, common signaling molecules control vascular and sympathetic axon growth, including the Nrp1 and plexin D1 receptors for Semaphorins (Sema) and the secreted guidance molecule Netrin1. Hence, one can speculate that these guidance molecules can also control vessel growth and arterial innervation and might influence tissue revascularization through both of these processes. Expression of p53 and Sema3E is enhanced in diabetic mice compared with normal mice. Consequently, neovascularization after VEGF treatment is poor in the ischemic tissues of diabetic mice, whereas treatment with VEGF plus plexinD1-Fc markedly improves neovascularization. Similarly, netrins accelerate neovascularization in an in vivo model of ischemia and they reverse neuropathy and vasculopathy in a diabetic murine model. The neuropeptide secretoneurin promotes proliferation and chemotaxis and reduces apoptosis in endothelial cells cultured under hyperglycemic conditions. In addition, secretoneurin activated ERK, eNOS, and especially AKT as well as EGF-receptor in hyperglycemic endothelial cells. Secretoneurin gene therapy promotes postischemic neovascularization in diabetic mice through stimulation of angiogenesis and arteriogenesis. Finally, nerve growth factor protects endothelial cells from apoptosis induced by T1D and facilitates reparative neovascularization.

**Diabetes Mellitus and BM-Derived Cell Recruitment**

The recruitment of EPCs expressing CXCR4 is controlled by hypoxia and the transcription factor HIF-1α, which activates the expression of CXCL12. Blockade of CXCL12 in the ischemic tissue prevents the recruitment of EPCs to the site of the tissue lesion. Conversely, transfection of ischemic mouse muscle with plasmids encoding for CXCL12α induces the recruitment of EPCs of medullary origin to the treated muscle. These effects involve activation of the VEGF-A-dependent and eNOS-dependent phosphatidylinositol 3’ kinase/AKT protein kinase. Diabetic mice showed impaired phosphorylation of BM eNOS, decreased circulating EPCs, and diminished CXCL12 expression in cutaneous wounds leading to impaired EPC recruitment. Diabetes mellitus also promotes the activation of the membrane-bound form of peptidyl peptidase-4 (DPP4), leading to reduced myocardial CXCL12 concentrations and resultant impairment in EPC recruitment. Overexpression of Ang-1 increases CXCR4/CXCL12 expression and promotes CD133+/c-kit+, CD133+/CXCR4+, and CD133+/CXCL12+ cell recruitment into ischemic diabetic hearts. Erythropoietin-producing human hepatocellular carcinoma (Eph) receptors and their ephrin ligands are key regulators of vascular development. EphB4 activation with an ephrin-B2-Fc chimeric protein increases the angiogenic potential of human EPCs. EphB4 activation enhanced P-selectin glycoprotein ligand-1 expression and EPC adhesion. Circulating diabetic mononuclear cells display a reduction in their adhesion and transmigration potential and pretreatment with ephrin-B2-Fc chimeric protein increases the adhesion and transmigration of circulating mononuclear cells restoring their proangiogenic potential in diabetic mice with CLI.

**Diabetes Mellitus and BM-Derived Cell Angiogenic Potential**

The function of bona fide EPC and different subset of angiogenic cells is also altered in diabetic subjects with peripheral arterial disease. Circulating angiogenic cells expressed higher level of mature miR-15a and miR-16. miR-15a/16 overexpression impairs healthy circulating angiogenic cell survival and migration. Conversely, miR-15a/16 inhibition improves diabetic cell defective migration, through upregulation of VEGF-A and AKT-3. Lower human tissue kallikrein protein levels are also observed in circulating angiogenic cells from T2D patients. Furthermore, the bradykinin type 2 receptor is normally expressed on T2D-circulating angiogenic cells but remains uncoupled from downstream signaling. Importantly, cotransfection with adenovirus encoding for tissue kallikrein protein and the bradykinin type 2 receptor rescues the diabetic defective phenotype. Interestingly, PHD2 mRNA levels are upregulated, whereas that of HIF-1α are downregulated in circulating cells from patients with CLI, and PHD2 silencing restores the therapeutic efficiency of BM-derived cells through upregulation of VEGF-A release. Diabetes mellitus also decreases the ability of adherent BM-derived mononuclear cells (BM-MNCs) to differentiate into cells with endothelial phenotype. Treatment with inhibitor of oxidative stress upregulates the number of EPC colonies derived from diabetic BM-MNCs. In the ischemic hindlimb model, injection of diabetic BM-MNCs isolated from antioxidant-treated or NOX2-deficient diabetic mice increases neovascularization when compared with untreated diabetic cells. Glucose-mediated EPC dysfunction is protein kinase C dependent, associated with reduced intracellular tetrahydrobiopterin (BH4) concentrations, and reversible after exogenous BH4 treatment. Subsequently, eNOS is uncoupled resulting in eNOS-mediated O2− production and impairment of EPC function in patients with diabetes mellitus. Notably, eNOS overexpression restores BM-derived cell angiogenic potential. This effect is associated with an increase in BM-MNC ability to differentiate into cells with endothelial phenotype in vitro and in vivo. Additional factors may be involved in the diabetes mellitus–induced EPC dysfunction. Notably, thrombospondin-1 mRNA expression is significantly upregulated in diabetic EPC, in relation with the decreased EPC adhesion activity in vitro and in vivo.
Molecular Mechanisms of Diabetes Mellitus–Induced Dysfunction of the Vasoactive Competence

Abnormalities in endothelial function are also linked to T1D and T2D. Endothelial dysfunction refers to inability of endothelium to properly regulate vascular tone, coagulation, and immune responses and essentially mentions a switch of the vascular homeostasis toward vasoconstrictive, proinflammatory, and thrombotic-related pathways. The most significant endothelium-derived mediator is NO and endothelial dysfunction is largely associated with reduced NO bioavailability, through multiple complementary mechanisms including decreased eNOS expression, eNOS uncoupling, and superoxide production of ROS. Both the activity of NADPH oxidase and the levels of NADPH oxidase protein subunits (p22phox, p67phox, and p47phox) are significantly increased in diabetic veins and arteries and participate in reduced NO levels and endothelial dysfunction. Interestingly, double transgenic mice with endothelial-specific insulin resistance and deletion of NADPH oxidase protein subunit Nox2 show decreased superoxide production and improved vascular function.

Induction of diabetes mellitus also results in oxidative loss of the tetrahdrobiopterin (BH4), a required cofactor for eNOS activity, forming BH2 and biopterin. Endothelial-targeted overexpression of the rate-limiting enzyme in BH4 synthesis, the guanosine triphosphate-cyclohydrolase I, reduces superoxide production from the endothelium and preserves NO-mediated vasodilatation in diabetic mice. Improvement in NO levels and endothelial function has also been unraveled in response to antioxidants such as superoxide dismutase. Alternatively, increase of potent vasoconstrictor including angiotensin II and endothelin foster vascular dysfunction in diabetic milieu. In this view, AT1-receptor blockade by telmisartan prevents downregulation of guanosine triphosphate-cyclohydrolase I and thereby eNOS uncoupling in experimental diabetes mellitus. In addition, telmisartan inhibits activation of superoxide sources, such as NADPH oxidase, mitochondria, and xanthine oxidase. Chronic endothelin type A receptor blockade increases vascular dilation by improving the release of dilator products by the endothelium. Interestingly, oral treatment of 4-week duration with the dual endothelin receptor antagonist, Bosentan, improves peripheral endothelial function in patients with type 2 diabetes mellitus. Finally, alteration in the mechanical properties of large compliance arteries may also fuel the pathogenesis of vascular dysfunction in diabetes mellitus. Hence, large arterial elasticity and fluid filtration across the arterial wall are impaired leading to decreased left ventricular–arterial coupling in diabetes mellitus. Interestingly, long-term treatment with aminoguanidine prevents AGEs accumulation on collagen and positively affects arterial wall properties in experimental diabetes mellitus.

Future Directions and Conclusions

Hyperglycemia has long been hypothesized to explain some of the effects of diabetes mellitus on cardiovascular complications. Alternatively, once glucose has entered the cell, most of it undergoes glycolysis. Three major enzymes regulate glycolysis: hexokinase, phosphofructokinase (PFK), and pyruvate kinase. It is tempting to speculate that glucose utilization and the glycolytic pathway in diabetic macro- and micro-environment might influence the activities of different type of cells involved in postischemic revascularization, including inflammatory and vascular cells. Interestingly, glycolysis has been shown to regulate vessel branching and blockade of PFK fructose-2,6-bisphosphatase 3 reduces endothelial cell proliferation and migration. When mice with the glucose transporter 1 overexpressing smooth muscle cells are subjected to femoral artery injury, an increased accumulation of neutrophils, upregulation of CCL2, and a reduction in vascular contractility are observed in the injured vessel. In addition, endothelial cells lining the blood vessel wall may also regulate lipid and glucose transport into diabetic tissues and affect the angiogenic response of the diabetic milieu. In this line, VEGF-B controls endothelial uptake and transport of fatty acids via a receptor complex composed of Nr1 and VEGFR1. VEGF-B blocking antibodies have been shown to prevent fatty acid transport and to increase endothelial glucose uptake, thereby restoring insulin sensitivity and improving glucose tolerance in rodent models of T2DM. Other cardiometabolic risk factors, including dyslipidemia, may also participate in the diabetes mellitus–induced vasculopathy. Indeed, a significant decrease in total n-3 polyunsaturated fatty acid, especially docosahexaenoic acid (DHA), is tightly coupled to diabetes mellitus. Interestingly, DHA-rich diet fully prevents retinal vascular pathology, leading to a concomitant suppression of tissue inflammation and correction of EPC number and function. Finally, endothelial cells not only are inert conduits delivering metabolites and oxygen but also establish a vascular niche that orchestrates regeneration and healing. Hence, a recent and elegant study uncovers that in response to liver injury, differential recruitment of proregenerative CXCR7-Id1 transcription factor versus profibrotic fibroblast growth factor 2 receptor type 1-CXCR4 angiocrine pathways balances regeneration and fibrosis. One can also hazard that diabetic endothelial cells are unable to deploy such paracrine trophogens, known as angiocrine factors, to stimulate regeneration.

Most current treatment options for reducing diabetes mellitus–related cardiovascular risk focus on controlling metabolic imbalances such as hyperglycemia, hypertension, and raised levels of free fatty acid. Although insulin is the obvious treatment of choice in T1D, first-line action for T2D is through lifestyle changes. During the past 15 years, therapeutic angiogenesis has emerged as an exciting salutary avenue to restore tissue perfusion in patients with diabetes mellitus with MI or CLI. However, the first generation of such angiogenic approaches mainly based on single growth factor shot or administration of angiogenesis-promoting cells from different origins showed limited benefits, precluding their use as a first-line treatment in these population of patients. Notably, we have been confronted with methodological caveats associated with dose, route of administration, frequency, timing, and delivery techniques. In addition, those previous strategies have mainly targeted the endothelium compartment. Thus, our efforts should now be directed toward the...
development of strategies of therapeutic revascularization, rather than therapeutic angiogenesis, using administration of a combination of specific factors or stem/progenitor cells targeting different types of vascular cells. Furthermore, the prevalence of cardiometabolic risk factors, including central obesity, insulin resistance, hypertension, and dyslipidemia, profoundly impairs endogenous and therapeutically induced revascularization. Hence, a deep understanding of the fine-tuning between vascular homeostasis and these cardiometabolic risk factors may benefit the positive outcome of patients with diabetes mellitus. Manipulation or inhibition of some of the described mechanisms may pave the way for the elaboration of second-optimized generation of revascularization therapies. In particular, targeting a combination of vascular and neural elements, using, for example, axon guidance molecules, may tackle vasculopathy and neuropathy and may thus constitute an accurate treatment for patients with diabetes mellitus with ischemic diseases. Alternatively, identifying molecular pathways orchestrating divergent angiogenic responses in the diabetic vascular niche may also value the development of therapeutic strategy that may ensure efficient vascular repair in the setting of diabetes mellitus. Finally, new vessel growth plays an ambiguous role when it comes to the pathogenesis of vascular disease in diabetes mellitus. Exacerbated vascularization occurs in diabetic retinopathy, nephropathy, and atherosclerosis, leading to increased risk of cardiovascular events. However, there is a clear deficit in new vessel formation in diabetic foot. Accordingly, impaired collateral growth leashes to the blunted myocardial perfusion are often observed in patients with diabetes mellitus. Hence, long-term and systemic strategies of therapeutic revascularization designed to restore tissue vascularization and perfusion should be carefully monitored in patients with diabetes mellitus and ischemic diseases.

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Disclosures

None.

References

depressed in op/op mice.

Am of collateral artery growth.


Chen XJ, Stinnett A. Ang-1 gene therapy inhibits hypoxia-inducible factor-1alpha (HIF-1alpha)-prolyl-4-hydroxylase-2, stabilizes HIF-1alpha expression, and normalizes immature vasculature in db/db mice. *Diabetes*. 2008;57:3353–3363.


induced impairment of postischemic neo
eral blood mononuclear cells from diabetic patients restore diabetes-
JJ, Guillausseau PJ, Silvestre JS, Lévy BI. Ephrin-B2-activated periph-
the proangiogenic potential of endothelial progenitor cells.
Leré-Déan C, Contreres JO, Sulpice E, Levy BI, Plouët J, Tobelem G, Le
infarcted hearts.
CD133+/c-kit+ cells and reduces myocardial apoptosis in db/db mouse
peptidase-4 modulates left v
Gallagher
Circulation
factor-1alpha enhances ischemic vasculogenesis and angiogenesis via vas-
Ceradini DJ, Gurtner GC. Homing to h
Nerve growth factor supplementation reverses the impairment,
neovascularization.
Franz WM, Kirchmair R. Secretoneurin gene therapy improves blood flow
Yokoyama M, Nojima A, Okada M, Koga H, Komuro I. Inhibition of
Circ Res
vival.

Madeddu P. Global remodeling of the vascular stem cell niche in bone
arrows of diabetic patients: implication of the microRNA-155/FOXO3a

Fadini GP, Sartore S, Schiavon M, Aliberti P, Sartore S, Schiavon M, Albiero M, Fadini GP. Diabetes causes bone marrow autonomic neuropathy and
impairs stem cell mobilization via dysregulated p66shc and sirt1.
A, Fadini GP. Diabetes causes bone marrow autonomic neuropathy
and impairs stem cell mobilization via dysregulated p66shc and sirt1.
A, Fadini GP, Sartore S, Schiavon M, Albiero M, Baesso I, Cabrelle A,

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9
62. Ceradini DJ, Gurtner GC. Homing to h
Nerve growth factor supplementation reverses the impairment,
neovascularization.
Franz WM, Kirchmair R. Secretoneurin gene therapy improves blood flow
Yokoyama M, Nojima A, Okada M, Koga H, Komuro I. Inhibition of
Circ Res
vival.

Madeddu P. Global remodeling of the vascular stem cell niche in bone
arrows of diabetic patients: implication of the microRNA-155/FOXO3a

Fadini GP, Sartore S, Schiavon M, Aliberti P, Sartore S, Schiavon M, Albiero M, Fadini GP. Diabetes causes bone marrow autonomic neuropathy and
impairs stem cell mobilization via dysregulated p66shc and sirt1.
A, Fadini GP, Sartore S, Schiavon M, Albiero M, Baesso I, Cabrelle A,

Howangyin and Silvestre
Diabetes Mellitus and Postischemic Revascularization
9
62. Ceradini DJ, Gurtner GC. Homing to h
Nerve growth factor supplementation reverses the impairment,
neovascularization.
Franz WM, Kirchmair R. Secretoneurin gene therapy improves blood flow
Yokoyama M, Nojima A, Okada M, Koga H, Komuro I. Inhibition of
Circ Res
vival.

Madeddu P. Global remodeling of the vascular stem cell niche in bone
arrows of diabetic patients: implication of the microRNA-155/FOXO3a

Fadini GP, Sartore S, Schiavon M, Aliberti P, Sartore S, Schiavon M, Albiero M, Fadini GP. Diabetes causes bone marrow autonomic neuropathy and
impairs stem cell mobilization via dysregulated p66shc and sirt1.
A, Fadini GP, Sartore S, Schiavon M, Albiero M, Baesso I, Cabrelle A,


**Significance**

Type 1 and type 2 diabetes mellitus are associated with micro- and macrovascular alterations resulting notably in coronary heart disease or peripheral arterial disease and increased morbidity and mortality. These vascular complications are characterized by both qualitative and quantitative adjustments of the vascular architecture and include abrogation of new vessel formation and remodeling of the pre-existing vasculature, as well as vascular dysfunction mainly depicted by functional disruption of the endothelium. The present review focuses on the molecular and cellular mechanisms that fuel the pathogenesis of vascular disease in the diabetic milieu and may participate in the negative outcome of patients with diabetic and myocardial infarction or critical limb ischemia.
Diabetes Mellitus and Ischemic Diseases: Molecular Mechanisms of Vascular Repair Dysfunction
Kiave Yune Howangyin and Jean-Sébastien Silvestre

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