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Review

Diabetes and vessel wall remodelling: from mechanistic insights to regenerative therapies

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Over the past two decades, extensive research has focused on arterial remodelling in both physiological and pathological ageing. The concept now describes the growth as well as the rearrangement of cellular components and extracellular matrix, resulting in either reduction or increase in vessel lumen. In diabetes, remodelling extends to capillaries, microvascular beds, and arteries of different calibre. This process is paralleled by accelerated atherosclerosis and accounts for an increased incidence of ischaemic complications. The incapacity of pre-existing and *de novo* formed collaterals to bypass atherosclerotic occlusions, combined with a decline in tissue capillary density, is responsible for the delayed recovery from ischaemia and ultimately leads to organ failure. The mechanisms of vascular remodelling are incompletely understood, but metabolic and mechanical factors seem to play an important role. Hyperglycaemia represents the main factor responsible for the fast progression of atherosclerosis as well as microangiopathy. However, intensive blood glucose control alone is insufficient to reduce the risk of macrovascular complications. Pharmacological control of oxidative stress and stimulation of nitric oxide release have proved to exert beneficial effects on vascular remodelling in experimental diabetic models. New approaches of regenerative medicine using vascular progenitor cells for the treatment of ischaemic disease have been shown to be safe and are now being tested for efficacy in preclinical and clinical trials.

1. Introduction

With the number of people with diabetes mellitus (DM) rising exponentially, the disease represents one of the greatest medical and socioeconomic challenges worldwide. Despite adapted treatment strategies, vascular complications represent the leading cause of morbidity and mortality in diabetic patients.[1] Therefore, nutritional and environmental interventions, together with new mechanistic therapies, are urgently needed to combat the new epidemics of ischaemic disease.

DM-associated vascular disease manifests with endothelial cell (EC) dysfunction, follows structural changes of large and small arteries with tissue hypoperfusion and hypoxia. These alterations recapitulate, in an accelerated version, the process of arterial remodelling with associated senescence that occurs with ageing.[2] In particular, diabetic subjects frequently show signs of accelerated atherosclerosis, undergo acute coronary syndromes, myocardial infarction with silent myocardial ischaemia, peripheral artery disease, and stroke.[3] Despite advances in interventional techniques, DM portends an adverse outcome following revascularization, and intimal hyperplastic remodelling still represents a common complication in diabetic patients.[4] Furthermore, DM impairs endogenous reperfusion mechanisms, i.e. activation of pre-existing arterial collaterals and generation of neo-vessels by arteriogenesis and angiogenesis, thereby worsening the recovery from an ischaemic insult.[5,6] This is aggravated by the concurrent development of microvascular complications. Limb muscle microangiopathy, together with peripheral neuropathy, is a key determinant in the pathogenesis of life-threatening foot ulcers, which affect 10% of diabetic patients. Proliferative retinopathy, a major cause of blindness, is present in more than 50% of patients with advanced type 1 DM. Nephropathy affects 35% of diabetic subjects and can evolve in chronic renal failure.[3,7]

Vessel integrity, once believed to be maintained exclusively by resident cells, is now recognized to be supported by bone marrow (BM)-derived endothelial progenitor cells (EPC).[8] Furthermore, local and BM-derived progenitor cells seemingly participate in re-endothelialization and arterial remodelling after vascular injury.[9] In both types of DM, vascular disease has been shown to be associated with a decline in EPC number and function. Common biochemical alterations affecting both mature EC and EPC might therefore concur in diabetic vascular complications.[10,11]

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This review illustrates the heterogeneous mechanisms of cellular and extracellular vascular wall remodelling, focusing on possible therapeutic targets for the prevention and treatment of diabetic complications.

2. Vascular remodelling

Remodelling affects capillaries and arteries of different calibre in both physiological (e.g. ageing) and pathological (e.g. atherosclerosis, hypertension, and diabetes) conditions. The term remodelling was originally coined for a complex set of vascular changes induced by chronic hypertension, including altered phenotype and function of EC and vascular smooth muscle cells (VSMC), as well as the extracellular matrix (ECM) structure and composition, leading to altered vessel wall-to-lumen ratio.[12,13] Principally, mechanical factors (wall shear stress, wall circumferential stress) and hypoxia determine hypertensive vascular remodelling.[14] In DM, metabolic factors, e.g. hyperglycaemia and oxidative stress, are important for microvascular remodelling. Interestingly, control of hyperglycaemia alone, while improving microvascular function, exerts only modest benefit on macrovascular complications.[15] This is in keeping with frequent association of DM with additive macrovascular remodelling risk factors, i.e. hypertension. In type 2 DM patients, concomitant hypertension causes enhanced inward remodelling of small arteries and attenuation of vessel dilation.[16] In this respect, specifically DM-associated vascular remodelling comprises chemical and biological modifications of the ECM, altered function of EC and VSMC, and changes in circulating cells/EC interaction via adhesion molecules, cytokines, and proteases (*Figure 1*). At the macrovascular level, these alterations cause the characteristic intima and media (IM) thickening that, along with increased stiffness and decreased vasomotion, are predictive for a high risk for cardiovascular events.[17]

2.1 Extracellular alterations

Increased IM thickness and vessel rigidity in type 2 DM patients result in an higher pulse wave velocity, decreased compliance, and luminal dilatation.[17] We will briefly focus on mechanisms responsible for wall stiffening, namely calcium and matrix protein deposition, increased protein glycoxidation, together with altered matrix degradation by matrix metalloproteinases (MMPs) and impaired VSMC relaxation. Calcification and increased expression of mediators of osteogenic differentiation of VSMC and pericytes are also seen in atherosclerotic plaques from non-diabetic patients;[18] altered insulin and glucose levels, however, accelerate this process.[19,20] Increased deposition of matrix proteins within the diabetic vessel wall was described several decades ago. Hyperglycaemia and subsequently advanced glycation end products (AGEs) were shown to increase basement membrane components and fibronectin expression in cultured EC.[21–23] The term AGE comprises a multitude of non-enzymatically glycosylated proteins and lipids with altered chemical and biological properties. Specifically, in plasma and locally at the site of vascular complications, increased glucose levels induce protein glycation. Early glycation products slowly degrade to form several different AGEs.[24] AGEs have emerged as key substances in diabetic vascular remodelling, mediating extracellular modifications, such as impaired ECM flexibility and increased matrix area by cross-linking of matrix proteins, e.g. through entrapment of molecules, such as low-density lipoproteins (LDL),[25,26] intracellular signalling, and cell–cell interaction (*vide infra)* (*Figure 1*). In addition, AGEs can reduce the activity of MMPs, a family of endopeptidases involved not only in matrix degradation, but also in cell migration, proliferation, and survival.[27,28] MMP activity is controlled at several levels, (i) gene expression, (ii) proteolytic activation of secreted pro-MMPs, and (iii) inhibitors (TIMPs) or activators (e.g. EMMPRIN). In the diabetic vessel wall, AGEs interfere with this system through alterations in activator protein 1 (AP-1) and transforming growth factor beta (TGF-β) signalling,[29,30] and specific expression of MMP subtypes.[31–33] Expression of MMPs and their regulators furthermore differs among different vascular cells in physiologic and disease conditions as well as in culture.[34–36] Altered cellular composition of the diabetic vessel wall (EC depletion, macrophage, and VSMC increased invasion/proliferation) aggravates the imbalance between different MMP (*vide infra*). In DM, differences in substrate specificity and altered expression/activity of individual MMPs might partly explain the increased plaque instability, the higher ECM volume and rigidity, and the reduced vascular healing capacity.

2.2 Intracellular alterations

2.2.1 Endothelial cells

Because of their incapacity to regulate glucose influx, EC represent a unique target for DM-induced damage. Protein glycation and excessive generation of reactive oxygen species (ROS) impair EC function and viability.[37] A more 'leaky' endothelial layer results in increased extravasation of plasma proteins. Among the mechanisms responsible for enhanced permeability is reduced density of tight and adherens junctions in diabetic EC due to decreased expression and increased destruction by proteases like μ-calpain.[38] In small vessels, also the loss of pericytes contributes to hyperpermeability (*vide infra*).

EC signalling is furthermore affected by glycation of ECM components, AGE receptor (e.g. RAGE) binding, and glycation/glycoxidation of intracellular proteins and transcription factors.[37,39] RAGE signalling and high intracellular glucose levels increase both mitochondrial and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent ROS generation (*Figure 2*).[40,41] While low ROS level is a physiological signalling mechanism for EC, e.g. promoting EC proliferation,[42] excessive ROS is cytotoxic, and contributes to impaired angiogenesis and diabetic cardiovascular complications.[43,44] In DM, ROS levels rise due to excessive superoxide production accompanied by inadequacy of scavenger mechanisms. Loss of the antioxidant enzyme glutathione peroxidase-1 aggravates atherosclerosis in diabetic mice, while administration of antioxidants rescues impaired endothelial function.[45–47] High ROS levels induce DNA strand breaks. Poly (ADP ribose) polymerase (PARP) is then activated in the attempt of repairing the DNA damage. In chronic hyperglycaemia, however, this protective mechanism is detrimental. PARP inhibits glyceraldehyde phosphate dehydrogenase (GAPDH), with consequent accumulation of

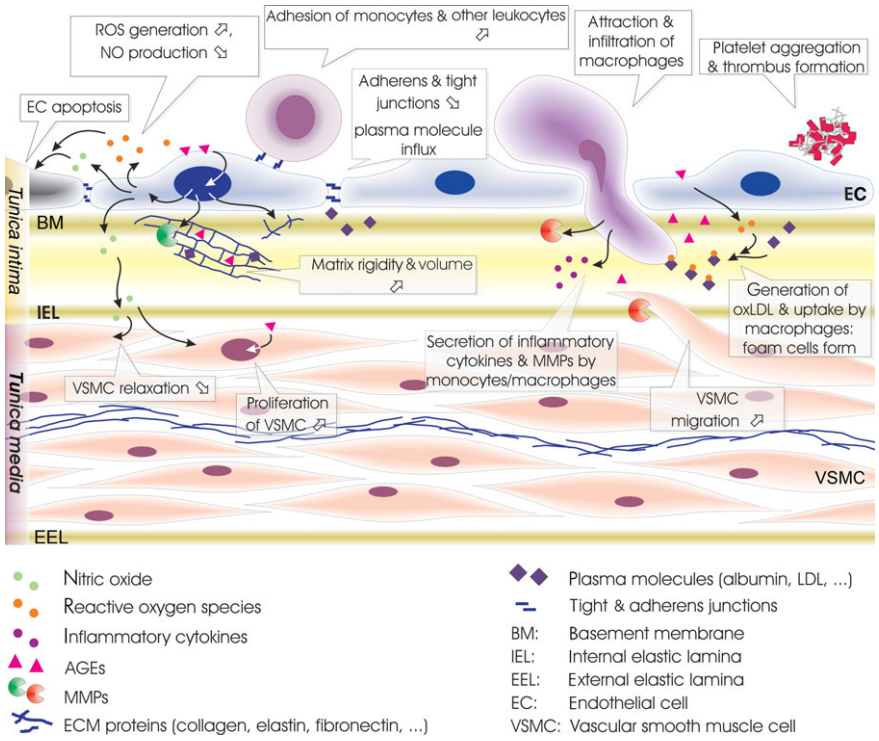

Figure 1 Alterations within intima and media of diabetic vessels. Endothelial cell apoptosis, reduced generation of nitric oxide (NO), and loss of endothelial cell junctions allow infiltration of macrophages and extravasation of plasma proteins. Leukocyte adhesion is facilitated by endothelial expression of adhesion molecules, aiding monocyte/macrophage infiltration and ultimately foam cell generation. Increased protein entrapment in the extracellular matrix, together with increased matrix deposition, and reduced degradation result in higher matrix volume. Low NO levels promote vascular smooth muscle cell (VSMC) proliferation and impede relaxation. Infiltrating macrophages and VSMC further increase intima/media (IM) thickness. Secretion of thrombogenic factors accelerates platelet adhesion and thrombus formation.

glucose by-products, which fuel AGE and diacylglycerol (DAG)/protein kinase C (PKC) pathways and thereby amplify ROS-induced endothelial damage (*Figure 2*).[48] Both high glucose and ROS promote EC apoptosis through nuclear factor-kappaB (NFκB) and c-Jun NH2-terminal kinase (JNK) pathways by caspase activation (*Figure 2*).[49,50]

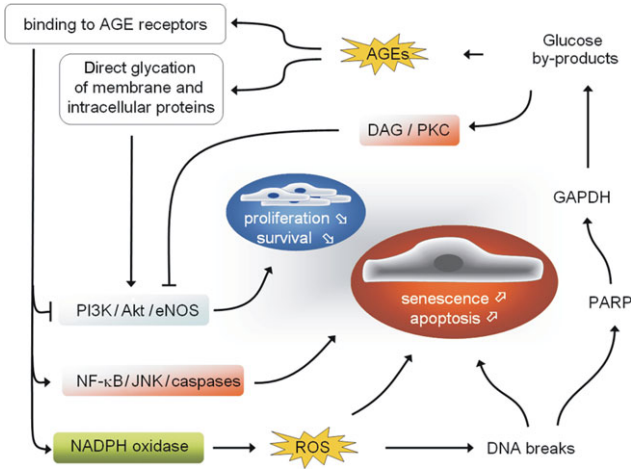

Figure 2 Reactive oxygen species (ROS) induced damage in diabetic endothelial cells. Reactive oxygen species-induced DNA strand breaks induce upregulation of poly(ADP ribose) polymerase (PARP) that in turn inhibits glyceraldehyde phosphate dehydrogenase (GAPDH). The resulting glucose by-products provide substrates for both advanced glycation end product (AGE) formation and for the diacylglycerol (DAG)/protein kinase C (PKC) pathway. AGE receptors activate intracellular signalling cascades leading to apoptosis through inhibition of PI3K/Akt/eNOS signalling and activation of the NF-κB and c-Jun NH2-terminal kinase (JNK) pathways as well as NADPH oxidase-dependent ROS generation.

RAGE activation stimulates a variety of intracellular signalling pathways, including the mitogen-activated protein kinase (MAPK) pathway via apoptosis signal-regulating kinase 1 (ASK1).[51] Interestingly, ASK1 activation causes transcriptional induction of plasminogen activator inhibitor-1 (PAI-1), a major inhibitor of fibrinolysis that may contribute to delayed resolution of thrombosis in diabetic patients.[52] Caspase activation is normally under the inhibitory control of the phosphatidylinositol 3-kinase (PI3K)/Akt/endothelial nitric oxide synthase (eNOS) axis.[53] PI3K signalling through Akt is a powerful pro-survival and pro-angiogenic mechanism, resulting among other effects, in the phosphorylation/activation of eNOS and nitric oxide (NO) generation.[54] In atherosclerosis, and especially within the diabetic vessel wall, eNOS is dysfunctional.[55] Besides reduced phosphorylation of eNOS at Ser^{1177}, due to decreased Akt activity, and eNOS inactivation via the DAG/PKC pathway, eNOS dysfunction is ascribed to oxidation of the cofactor tetrahydrobiopterin (BH_4), a mechanism referred to as 'eNOS uncoupling'.[56] Uncoupled eNOS produces oxygen radicals instead of NO, adding to the increased oxidative stress and to reduced NO levels.

2.2.2 Vascular smooth muscle cells

Situated within ECM with enhanced rigidity and beneath a layer of EC, which fail to provide appropriate levels of vasoactive substances,[57] diabetic VSMC meet strong counteracting conditions. Those external factors are accompanied by VSMC failure to respond to vasoactive stimuli.

Although VSMC are exposed to the same cues as EC, namely high glucose and oxidative stress, their response is the opposite with regard to proliferation and migration capacities, contributing to the disease-associated progressive atherosclerosis and restenosis.[58,59]

The signalling cascades involved are similar in VSMC and in EC, including ROS and AGE generation increase, mediated by RAGE, NADPH oxidase, PKC, and NFκB pathways combined with reduction in PI3K/Akt/eNOS signalling. Both, insulin and glucose enhance the proliferative ability of cultured VSMC and reduce apoptosis,[60] but the underlying mechanisms are still not fully understood. *In vitro*, high glucose down-regulates PKC-β in VSMC, resulting in the activation of proliferation.[61] Other PKC isoforms, however, show different behaviour: PKC-α up-regulation by hyperglycaemia is responsible for the increased expression of growth factors (GFs) and their receptors, such as TGF-β and TGF-β receptor-1.[62] PKC signalling is also implicated in hyperglycaemia-mediated reduction of VSMC apoptosis.[63]

VSMC contractility and survival are also controlled by the renin–aldosterone–angiotensin system, with its effector angiotensin II (AngII). High glucose enhances AngII response, through up-regulation of the AngII AT1 receptor, and enhanced ROS production.[64,65] Activation of extracellular signal-regulated kinases (ERK)1/2, JNK, and p38 MAPK by AngII is enhanced by high glucose, and in turn results in AngII up-regulation in VSMC.[65,66] Other signs of vascular remodelling attributed to AngII comprise vessel wall calcification, enhanced permeability, and monocyte infiltration. Increased VSMC migration in DM is furthermore attributed to RAGE-mediated up-regulation of cytokines and GFs, e.g. TGF-β1, platelet-derived growth factor (PDGF), and tumour necrosis factor α.[67] Digestion of the internal elastic lamina by MMPs furthermore facilitates VSMC migration/invasion of the intimal layer.

2.2.3 Pericytes/podocytes

In microvessels, EC and basal lamina are surrounded by a discontinuous layer of pericytes in direct contact with EC through gaps in the basal lamina. A similar position is maintained by the podocytes in renal glomeruli. These cell types through both direct cell–cell contact and paracrine signalling, regulate EC survival, proliferation, and migration, and stabilize nascent neovessels during angiogenesis. Pericytes share characteristics with VSMC, e.g. contractility in response to vasoactive stimuli, and *in vitro* studies suggest that they can give rise to VSMC. However, it is still unclear whether pericytes are VSMC precursors, or both independently derived from a common progenitor, with pericytes maintaining higher plasticity.

Under physiologic conditions oxygen induces pericyte contraction. High levels of oxidative stress, however, evoked by high glucose and AGEs, induce pericyte and podocyte apoptosis through mechanisms similar to those in EC. For example, forkhead box transcription factors, known mediators of apoptosis, are activated upon p38 activation and Akt dephosphorylation in the presence of glycated collagen.[68] These effects are amplified by loss of insulin-mediated pro-survival signalling.[69] Moreover, high glucose up-regulates phagocyte-type NAD(P)H oxidase in pericytes increasing ROS production.[70] Increased MMP-2 activity and reduced TIMP3 expression in DM concur in promoting pericyte apoptosis via detachment from the matrix.[71–73] Similar to pericytes, podocytes are lost in renal glomeruli as a consequence of ROS-induced apoptosis.[74] Furthermore, modulation of ion channel activity, e.g. $P2X_7$ purinoceptors, might contribute to accelerated pericyte/podocyte death, as recently postulated.[75–77] Pericyte impairment, as in the diabetic retina, leads to uncontrolled growth of immature and 'leaky' vessels, easily broken causing haemorrhagic damage and vision loss.

2.3 Cell–cell interactions

Diabetes-associated EC dysfunction facilitates vascular inflammation via GF and cytokine secretion, and adhesion molecules expression.[78] Increased leukocyte affinity to EC has been linked to the pathogenesis of diabetic microangiopathy and atherogenesis.[79,80] Furthermore, facilitated leukocyte *trans*-endothelial migration (TEM) due to increased endothelial permeability and cytokine/GF generation contributes to IM thickening and plaque instability, symptomatic for diabetic atherosclerosis.[81,82] High insulin and glucose levels increase adhesion molecule expression on leukocytes and EC, assisting initial rolling and later firm adhesion preliminary to TEM.[83,84] As discussed before, both AGEs and AngII activate transcription factors (NF-κB and AP-1) in a ROS-dependent manner, inducing adhesion molecule gene expression.[85] Consistently, RAGE signalling inhibition, NF-κB inactivation, and ROS scavenging reduce monocyte adhesion to the endothelium and retard the development of vasculopathies in diabetic patients.[86–88] Excessive thrombus formation in diabetic patients is attributed at least in part to AngII-mediated up-regulation of PAI-1 in response to RAGE activation. Furthermore, glycoxidation of diabetic platelet cell membrane proteins is associated with accelerated aggregation and decreased sensitivity towards aspirin.[89,90] Finally, AGEs in the *trans*-endothelial space induce the production of chemoattractants for monocytes.[86] This process is auto-amplifying, since activated monocytes that infiltrate the vessel wall produce ROS and inflammatory cytokines, which in turn stimulate ROS generation in VSMC. The above-described mechanisms of DM-induced atherogenesis follow the pattern observed during arterial ageing,[91,92] indicating common mechanisms for arterial remodelling, e.g. eNOS uncoupling and systemic insulin resistance.[93,94]

3. Impairment of angiogenesis and vasculogenesis

Clinical and experimental evidence indicates that altered remodelling of arterial collaterals as well as *de novo* vascularization play a key role in impaired recovery from ischaemia in DM. We and others used a model of severe hind limb ischaemia to investigate the cellular and molecular mechanisms of disturbed angiogenesis in diabetic animal models.[6,95]

3.1 Angiogenesis inducers and inhibitors

Both forms of diabetes feature an insufficient surge of endothelial GFs at sites of ischaemia, namely members of the vascular endothelial GF (VEGF) and insulin-like GF families, hindering reparative neovascularization.[6,96] Impaired VEGF signalling translates into reduced monocyte chemotaxis to sites of ischaemia, where those cells are putatively implicated in the formation of new arterial collaterals.[97]

Studies from Tanii *et al.*[98] contradict, however, the primary involvement of VEGF-mediated mechanisms.

Microangiopathy and peripheral neuropathy often develop concomitantly and aggravate each other. The neurotrophin nerve growth factor (NGF) is produced by EC, which also express NGF receptors.[99] In DM, impaired NGF signalling together with overexpression of the neurotrophin-related death receptor p75 increases EC apoptosis and impairs wound healing.[100,101] Other neuropeptides from sensory neurons are implicated in angiogenesis and their deficit could participate in the delayed repair of diabetic ulcers.[102]

Disequilibrium of angiogenesis promoters and inhibitors can lead to exuberant but dysfunctional neovascularization, as seen in the diabetic retina, as well as vascular destabilization, as observed in skeletal and cardiac muscle, thus supporting a high degree of heterogeneity of diabetic vascular pathology.[103,104]

3.2 Vasculogenesis: dysfunction of diabetic endothelial progenitor cells

Neovascularization accomplished with contribution of stem cells (SC) and BM-derived EPC, termed post-natal vasculogenesis, is a multi-step process cooperating with regeneration facilitated by resident vascular cells (*Figure 3*). Circulating EPC from type 2 DM patients are numerically and functionally altered and correlate inversely with levels of haemoglobin A_1C and cardiovascular risk factors.[10] The finding of an association between EPC dysfunction and cardiovascular complications is important but not sufficient to draw pathogenetic conclusions. Furthermore, the specific location and mechanisms of EPC damage and reduction remain unknown. The initial stage of vasculogenesis is represented by BM–SC activation and transmigration to the central BM 'vascular niche'.[105,106] BM–SC express high levels of antioxidant enzymes.[107] Not surprisingly, therefore, oxidative stress does not play a major role in high glucose-induced EPC dysfunction.[108] A recent report from Li *et al.*[109] indicates that circulating EPC number in diabetic mice is significantly reduced with arterial injury; however, the number of EPC in BM in diabetic mice was greater. This important observation underlines the possibility that DM-induced EPC damage may occur after liberation from the BM, with exposure to high glucose. Those findings also lead us to speculate that the EPC increase in BM of diabetic animals may represent a compensatory mechanism for increased mortality of those cells in the circulation.

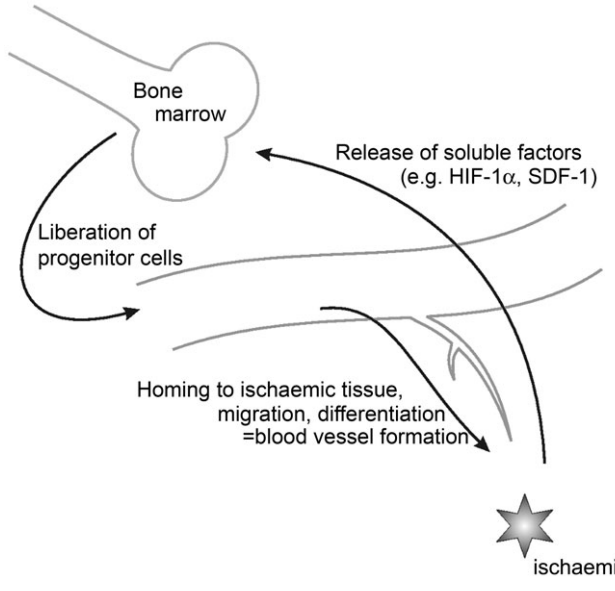

Figure 3 Ischaemia-induced vasculogenesis. In response to ischaemia, secreted soluble factors, like hypoxia-inducible factor-1α (HIF-1α) and stromal cell-derived factor-1, induce proliferation, differentiation, and liberation of vascular progenitor cells from the BM. In the tissue, EPC adhere to the vessel wall and migrate along gradients of chemotactic factors (e.g. VEGF, SDF-1).

We previously showed that moderate increase in glucose levels impairs cell cycling and migration and increases apoptosis of cultured human EPC.[36] Consistently, human diabetic $CD34^+$ progenitor cells show altered migratory ability towards stromal cell-derived factor-1 (SDF-1).[110] Furthermore, diabetic EPC show impaired integrative capacity in neovasculature of ischaemic organs and reduced re-endothelialization ability after arterial injury.[109] Recent evidence shows that eNOS uncoupling is central in EPC mobilization and function in humans as well as in a DM animal model.[111,112] Elevation of asymmetric dimethylarginine, an endogenous NOS inhibitor, in DM, could contribute to this phenomenon.[113] Glycoxidated proteins are suspected for the reduced availability and dysfunction of EPC in DM, as *in vitro* studies showed that activation of the Akt/p53/p21 pathway in healthy EPC cultured in the presence of oxidized small and dense LDL (ox-dmLDL), results in a senescent-like growth arrest.[114,115] Apart from endogenous liabilities, diabetic EPC release unidentified factors that accelerate microvascular EC ageing.[10,11] The mobilization of SC from BM to sites of injury is considered instrumental to the repair and stabilization of vascular damage.[18] Initial evidence suggests however that DM could convert this mechanism into an adverse process, with recruitment of pro-inflammatory progenitors prevailing on those endowed of regenerative potential. In addition to SC from distant sources, local adventitial progenitor cells may contribute to vascular remodelling. Following vascular injury, those progenitor cells migrate into the intima and differentiate into smooth muscle cells[116] but are also capable of participating in the formation of peri-adventitial vascular sprouts, thus establishing the basement for arterial collateralization.[117] Thus, adventitial progenitor cells might play a Dr Jekyll–Mr Hyde role in the development of arteriosclerosis, angioplasty-induced restenosis, vein graft atherosclerosis, and reparative vascular growth.

4. Regenerative therapies

New mechanistic insights in the pathogenesis of endothelial dysfunction were rapidly translated into new therapeutic opportunities. Among emerging strategies, ROS and AGE scavengers, PKCβ inhibitors,[118–121,122] and potentiation of eNOS activity with BH_4, statins, and thiazolidinediones (glitazones) reportedly alleviate endothelial dysfunction in diabetic animals.[123,124] Clinical trials demonstrated the ability of statins and glitazones to reduce the incidence of cardiovascular events such as myocardial infarction and stroke in diabetic patients (see Hamilton *et al.*[125] for review).

In this final section of this review, we will concentrate on new approaches of regenerative vascular medicine, namely therapeutic angiogenesis and SC therapy. Based on the hypothesis that VEGF signalling is decreased in the diabetic

heart, Yoon *et al.*[126] injected a plasmid DNA encoding $VEGF_{165}$ in the myocardium of diabetic rats, thereby restoring microvascular homeostasis and preventing heart failure development. In addition, VEGF gene therapy proved to stimulate angiogenesis in ischaemic limbs of diabetic mice.[6] It was argued that the short duration and leaky neovascularization induced by this GF may be worrisome in diabetes. Furthermore, different VEGF isoforms and splice variants have been shown to induce different signalling mechanisms, suggesting better contemplation before clinical usage of this protein. There has been, in general, a disappointing outcome regarding angiogenesis in clinical trials,[127] with only recombinant human PDGF-BB being clinically approved for the treatment of diabetic neuropathic ulcers, yet none for ischaemic ulcers.[128] This claims for the introduction of pleiotropic angiogenic agents able to address the multi-factorial determinants of diabetic endotheliopathy. Our group previously showed that gene therapy with human kallikrein prevents endothelial dysfunction and microangiopathy in limb muscles of mice with type 1 DM.[95,129,130] Several mechanisms of kallikrein action in this setting are conceivable, including improvement of ECM flexibility by its protease function and increased kinin release. With superimposed limb ischaemia, kallikrein promotes arterial collateralization through generation of kinins and NO, but independently of VEGF.[131] The kinin pathway has furthermore been shown to mediate the pro-angiogenic effects of angiotensin-converting enzyme inhibition in DM.[104]

Schatteman and colleagues[132] first analysed the role of EPC in DM-related microangiopathies. $CD34^+$ circulating cells from type 1 DM patients produced fewer EC per ml of blood and exogenous non-diabetic $CD34^+$ cells accelerated blood flow recovery in a diabetic model of limb ischaemia. Moreover, heterologous transplantation of non-diabetic BM-derived progenitor cells promotes vasculogenesis and wound healing in type 2 DM mice, whereas homologous, diabetic progenitor cells favour cicatrization but inhibit vasculogenesis.[133] Thus autologous SC therapy for diabetic vascular regeneration may have limitations both intrinsic in progenitor cells and imposed by the diabetic environment. Furthermore, differences may exist with regard to EPC function and curative properties in type 1 and 2 DM. Finally, a crucial point determining the therapeutic benefit of EPC is represented by their ability to secrete pro-angiogenic factors, which may be diminished or substituted by inflammatory cytokines in diabetic EPC.

Future directions include attempting to rescue those functional defects and improving EPC recruitment/engraftment.[134] One candidate is NO, which is a common mediator of intracellular pathways in EC and EPC (*Figure 4*). Recent studies indicate statin and, to an even greater extent, NO-donating statins potently stimulate reparative angiogenesis and arteriogenesis in type 1-DM.[135] Of note, statins improve the migratory and survival capacity of EPC via the PI3K/Akt/eNOS axis and NO-donating statins further amplify these effects.[135]

5. Summary

EC and EPC dysfunction pairs with a complex set of cellular and structural modifications within the vessel wall leading to diabetic vascular complications. We believe that EPC will have a central role in new targeted therapies for diabetes angiopathies, but more data establishing their nature and function are to be achieved.

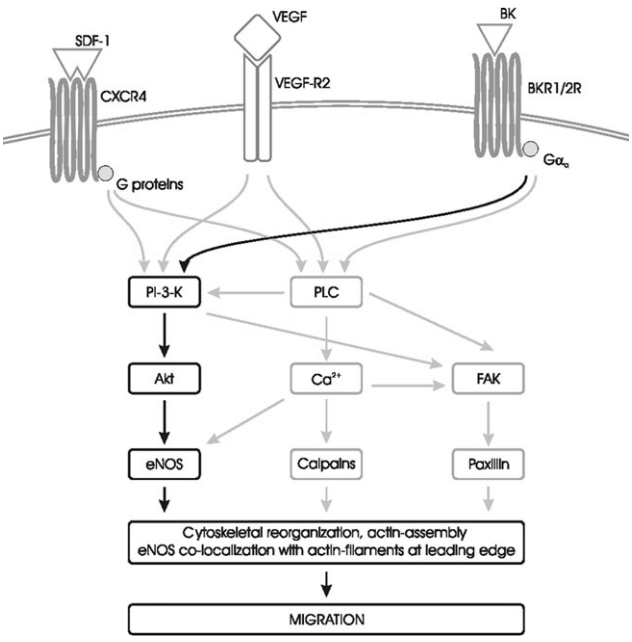

Figure 4 Intracellular signalling pathways for migration. Migration can be activated by heterogeneous chemokine gradients through a redundant array of receptors. Two major classes of receptors are implicated: G protein-coupled receptors and tyrosine kinase receptors. PI3K and phospholipase C (PLC) activation of the Akt/eNOS pathway increases intracellular calcium and induce focal adhesion assembly, thus mediating cytoskeletal reorganization. BK, bradykinin; BKR1/2R, bradykinin receptor 1 or 2; FAK, focal adhesion kinase.

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