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Glucose-Induced Cellular Signaling in Diabetic Retinopathy

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Contents

Keywords Diabetes • Retinopathy • Complications • Endothelial cells • Pericytes • Angiogenesis • Extracellular matrix • Cellular signaling

INTRODUCTION

Diabetic retinopathy (DR) is a microvascular complication of diabetes. It is the most common cause of blindness in the working population. Nearly all people with diabetes, both type 1 and type 2, will eventually develop some form of retinopathy [1]. Clinical trials have consistently shown that good glycemic control can reduce the development of retinopathy in both type 1 and type 2 diabetic patients [2, 3]. Other factors such as hyperlipidemia and hyperinsulinemia may also be involved. However, the major contributor does seem to be excess blood glucose levels. Sustained hyperglycemia leads to a sequence of adverse events in the retina (summarized in Fig. 1). Early events include altered expression of vasoactive factors and basement membrane (BM) proteins [4–6]. This manifests as loss of vasoregulation, thickening of the BM, and increased permeability. Increased permeability may also cause macular edema and significant vision loss. With continued hyperglycemic insult, the vascular cells exhibit exhaustion and degeneration leading to the formation of acellular capillaries [7–9]. All these functional and structural changes then converge to create an ischemic retina. Elaboration of growth factors to induce new blood vessel formation then proceeds. This sequence of events, continued insult, and continued adaptation, ultimately causes unregulated angiogenesis

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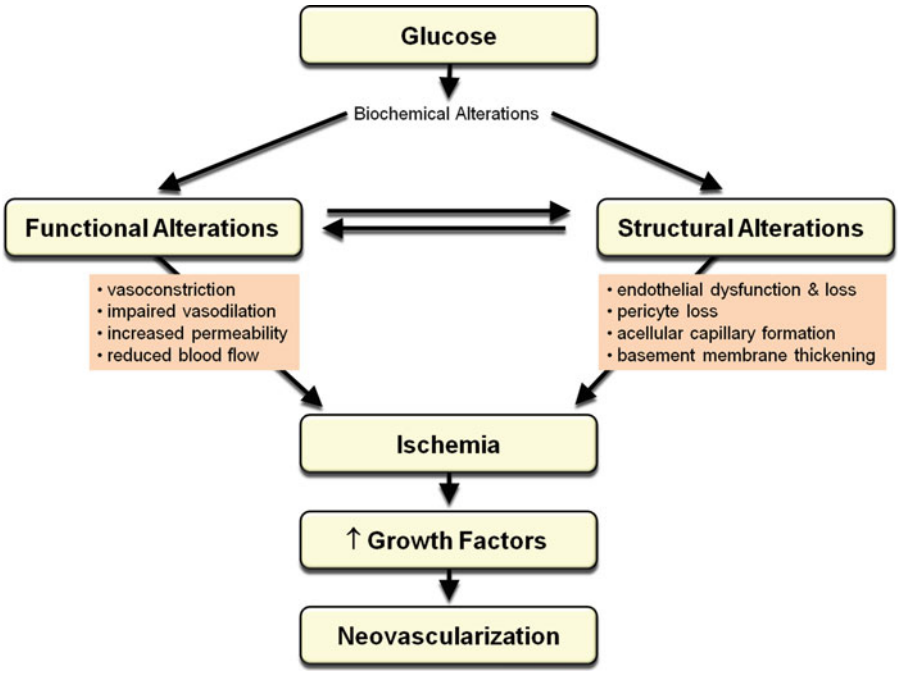

Fig. 1. Key events in the development and progression of DR. High plasma glucose levels lead to biochemical dysfunction in the retinal vascular cells. These changes result in structural and functional alterations at the vascular unit level. Reduced blood flow to the retina produces an ischemic environment which dictates elaboration of various angiogenic factors. These continued insults to the retinal tissue ultimately lead to EC hyperplasia and unregulated angiogenesis.

and blindness in diabetic patients. It is well accepted that understanding the molecular basis of endothelial cell (EC) dysfunction and loss will provide better therapeutic targets for DR. In this chapter, we review the cellular and molecular (signaling) mechanisms that ultimately lead to the development of DR.

CELLULAR TARGETS IN DR

In order to gain insight into the pathogenetic mechanisms underlying any disease, the first step is to develop in vitro and in vivo models that provide a phenocopy or at least exhibit the key structural and functional features of the disease. A prerequisite, therefore, is to identify the target cellular population. In the case of DR, retinal fluorescein angiography has provided important information about the primary cellular target [10, 11]. These studies show numerous areas of nonperfusion in the retina. The underlying cause of nonperfusion seems to be loss of vascular cells [12, 13]. These vascular cells include both ECs and pericytes that eventually succumb to glucotoxicity.

Endothelial Cell (EC) Dysfunction

Retinal angiography and digest studies show that normal retinal vascular perfusion is dependent on intact endothelium [14, 15]. The working hypothesis is that high levels

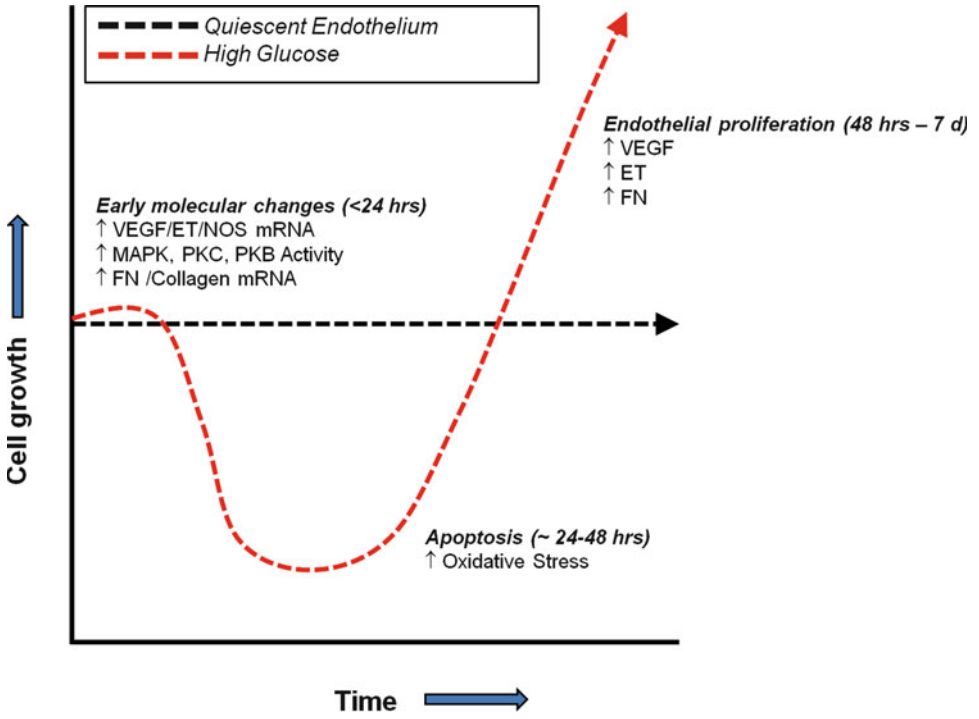

Fig. 2. Molecular and phenotypic changes in ECs exposed to high levels of glucose. Studies from our labs and others have shown that acute exposure to high glucose causes reduced viability and increased apoptosis in the ECs. However, with continued exposure, the ECs proliferate which is associated with increased matrix protein and VEGF production. *ET* endothelin; *FN* fibronectin; *MAPK* mitogen-activated protein kinase; *NOS* nitric oxide synthase; *PKB* protein kinase B; *PKC* protein kinase C; *VEGF* vascular endothelial growth factor.

of glucose lead to EC dysfunction and loss. An important assumption, therefore, is that high levels of extracellular glucose equate to high levels of intracellular glucose. In other words, there is no adaptive transport mechanism in the ECs. This certainly seems to be the case. ECs incorporate glucose via facilitative diffusion without significant alterations of glucose transporter-1 (Glut1) levels [16]. Therefore, continued exposure of ECs to high glucose leads to continued intracellular glucose accumulation. When assayed in culture, exposure of ECs to high glucose causes activation and dysfunction which is reflected by increased extracellular matrix (ECM) protein production and altered cellular activities [17–22]. Data from our laboratories and others show that this simple in vitro model illustrates most of the molecular changes that we see in clinical DR (Fig. 2). Early changes in the ECs following glucose exposure include reduced viability and increased apoptosis [23]. Interestingly, these changes are followed by increased proliferation [24]. This biphasic effect is reminiscent of EC changes in the early and advanced DR. Although the mechanism of this biphasic effect is not clear, we hypothesize that the mechanism of the late proliferative response is a change in the microenvironment—this is expected to occur in vivo as well. ECs rest on a scaffold of ECM proteins called the BM. This matrix serves as a reservoir of growth factors and other signaling proteins. With continued exposure to

high glucose levels, the ECs may accumulate growth factors and other mitogens in the matrix. In fact, ECs exposed to glucose for more than 72 h have been shown to increase protein levels of an EC-specific mitogen, vascular endothelial growth factor (VEGF) [25]. We have also shown that the mRNA of VEGF is upregulated as early as 24 h following exposure to high levels of glucose [26]. In addition, the matrix itself is expected to change in terms of the protein amount and the protein composition (see below). This potentially creates a permissive environment that mediates the late changes of glucose in culture and in advanced clinical DR.

Endothelial-Pericyte Interactions

Pericytes are the contractile cells present in microvessels (similar to smooth muscle cells in larger vessels). These cells are in close contact with the ECs and form a discontinuous layer. The physiological function of the pericytes is to stabilize vessels, regulate vessel contraction, and keep the endothelium in a quiescent state. This intimate relationship between the vascular cells suggests that aberration in one cell type will lead to alterations in the phenotype of the other cellular component. However, when pericytes are cultured in high levels of glucose, we see an interesting contrast to ECs. Both pericytes and smooth muscle cells exhibit an autoregulatory glucose transport mechanism [16], that is, exposure to glucose leads to downregulation of Glut1. The overall transport of glucose seems to be higher in pericytes possibly due to greater biosynthetic ability. Therefore, these perivascular cells also undergo glucose-induced dysfunction and loss. In fact, loss of pericytes is considered one of the structural hallmarks of DR [7–9]. Pericyte loss is implicated in contributing to acellular capillary formation and may also be important in late stages of DR. Evidence for this comes from studies in platelet-derived growth factor-B knockout mice that lack pericytes in the brain capillaries [27]. These animals develop microaneurysms, acellular capillaries, and EC hyperplasia. These results are exacerbated when PDGF-deficient animals are made diabetic [12] suggesting an important role of pericyte-EC interaction in advanced DR.

The biochemical mechanisms underlying pericyte loss seem to be similar to ECs with the same players emerging (metabolic distress, vasoactive factors, protein kinase activation). In addition, it has been shown that an abrupt drop in glucose levels causes pericyte apoptosis [28]. Another mechanism may involve the angiopoietin system. Hyperglycemia has been shown to increase the expression of angiopoietin-2 in the retina that leads to pericyte dropout [29]. Furthermore, angiopoietin deficiency in the diabetic animals prevented pericyte loss and subsequent acellular capillary formation.

Endothelial-Matrix Interactions

Neovascularization, formation of a complete vascular unit either through angiogenesis or vasculogenesis, is a multistep process. Both endothelial and perivascular cells undergo a number of structural and functional changes to form a blood vessel. These cellular activities include endothelial proliferation and migration, formation of cell-cell contacts and tubules, recruitment of pericytes, and contribution to the ECM. In addition to providing a scaffold for the organization of the vascular cells, the ECM has been implicated in providing critical cues for proper blood vessel formation [30, 31]. The BM (sheet of ECM proteins) of normal microvessels predominantly contains laminin,

collagen, and nidogen (entactin) [32]. A consistent feature of DR is (a) an increase in the ECM proteins; (b) a switch in the type of ECM proteins, that is, composition; and (c) posttranslational modifications of ECM proteins such as glycation.

In cultured retinal ECs, high levels of glucose can increase mRNA expression of both collagen and fibronectin (FN) [19, 33, 34]. The retinal BM of diabetic animals also shows increased expression of collagen, laminin, and FN [35]. These are early molecular changes and are evident in approximately 8 weeks following diabetes induction [35]. We have previously shown that FN is upregulated in the retinal tissues of diabetic rats in 1 month [36]. This increased expression continues for up to several months. The upregulated matrix protein expression then manifests as thickening of the BM in animal models [37]. In addition to collagen and FN, tenascin has been found in retinal vessels of diabetic patients and animals [38, 39]. It is important to note that this does not represent a general phenomenon of BM duplication/expansion but is a selective upregulation of key ECM proteins. For example, no difference in the amount of proteoglycans in diabetic patients has been reported [40]. This suggests that the composition of the BM may be important in providing critical cues to the vascular cells [30–32, 41, 42]. In support, we have recently shown that FN undergoes alterative splicing in DR to produce an embryonic isoform, ED-B + FN (also known as oncofetal FN) [26, 43]. Increased levels of this isoform are evident in vitreous of patients with advanced DR [43, 44] and retinal tissues of diabetic rats [43]. In cultured vascular ECs, we have shown that ED-B + FN is increased following exposure to high levels of glucose and that this FN isoform is involved in VEGF expression and EC proliferation.

Functionally, FN in the matrix may play a critical role in DR. FN is highly expressed in developing vessels as compared to stable quiescent vessels [45, 46]. During vascular remodeling (e.g., during wound healing or tumorigenesis), FN is upregulated [47, 48]. Further support of a functional role of FN in the retina comes from studies that show expression of FN in the active zones of vascularization [49]. FN also provides critical survival and proliferative signals to brain capillary ECs [50]. ECs express a number of ECM protein receptors, and function-blocking antibodies against FN integrins lead to reduced EC proliferation [50].

SIGNALING MECHANISMS IN DR

Altered Vasoactive Factors

DR is a culmination of numerous biochemical alterations that take place in the vascular tissue of the retina. An important physiological function of the endothelium is the regulation of regional blood flow. This is achieved by creating a balance between vasoconstricting factors and vasodilating factors. Diabetes leads to a disruption of this balance, and these altered vasoactive molecules play a role in both the early and the late stages of DR. Increased vasoconstriction and impaired endothelium-dependent vasodilation has been reported in diabetes [37, 51–55]. This vasoregulatory impairment has been shown to precede the structural changes in the vasculature [52, 54, 56–59]. The mechanistic basis of impaired endothelium-dependent vasodilatory responses has been extensively researched in diabetic patients, animal models, and cultured cells. This mechanism involves increased expression of endothelin-1 (ET-1), the most potent vasoconstrictor

[60]. ETs are short peptides that are secreted by ECs and mediate vasoconstriction by binding to ET receptors on the perivascular cells. Increased ET has been shown to cause vasoconstriction and reduced blood flow in diabetes [60]. Interestingly, improvement of the vasodilator responses have also been noted in diabetic patients that were administered an ET receptor antagonist [61]. In streptozotocin-induced diabetic rats, we have reported that diabetes-induced retinal capillary vasoconstriction is normalized with an ET receptor antagonist (Bosentan) [37]. We have also shown that high levels of glucose increase ET-1 and mediate increased EC permeability and ECM protein expression in cultured cells [26, 62, 63]. ET may also function as a mitogen for both perivascular cells [64, 65] and ECs [66, 67] which may be important in the late stages of DR.

It is expected that increased ET-1 levels may accompany decreased vasodilator levels (such as nitric oxide; NO). NO is produced by a family of enzymes called NO synthases (NOS). Studies have shown increased levels of both endothelial (e-) and inducible (i-) NOS enzymes in response to high levels of glucose [68–71]. This is also seen in animal models and human diabetes [69]. A number of signaling pathways that are activated in diabetes may lead to increased expression of NOS. These pathways may include VEGF [72] and protein kinase pathways [72–74]. The reason for this apparent discrepancy has been recently hypothesized to be an increased scavenging and reduced bioavailability of NO. In diabetes, NO levels may be reduced through sequestration by reactive oxygen species (ROS). It is also important to note that increased NOS expression may not lead to increased NO production. Acute exposure of ECs to glucose decreases NO generation by agonists including bradykinin [75]. These effects were shown to be the direct result of high glucose levels. Purified eNOS, when assayed in the presence of glucose, shows significantly lower NO production [75]. This suggests that increasing NO production/availability may undo some of the glucose-induced changes. When diabetic animals are treated with an NO donor, molsidomine, the diabetes-induced vasoconstriction in the retina is normalized [76].

Alteration of Metabolic Pathways

Polyol Pathway

Physiologic metabolism of glucose is accomplished mainly by the glycolytic pathway. However, in diabetes, increased flux and shunting of glucose through alternative pathways take place (Fig. 3). One such pathway is the polyol pathway [77, 78]. In this pathway, glucose is metabolized to sorbitol by aldose reductase (AR) [78]. Sorbitol itself may cause cellular damage [78, 79] which may be prevented by myo-inositol supplementation [80]. However, the major contribution of the polyol pathway to the adverse effects of high glucose levels seems to be the alteration in enzyme cofactor levels. The first enzymatic reaction that converts glucose to sorbitol requires NADPH. An increase in glucose flux is expected to decrease NADPH levels. NADPH is also a cofactor for antioxidant enzyme system (reduced glutathione) and, therefore, contributes to impairment of cellular antioxidant system. The second reaction that converts sorbitol to fructose requires NAD+ and generates NADH. It is believed that increased NADH production leads to augmented levels of glyceraldehyde 3-phosphate. Increased glyceraldehyde 3-phosphate may then increase advanced glycation end product formation through methylglyoxal [81].

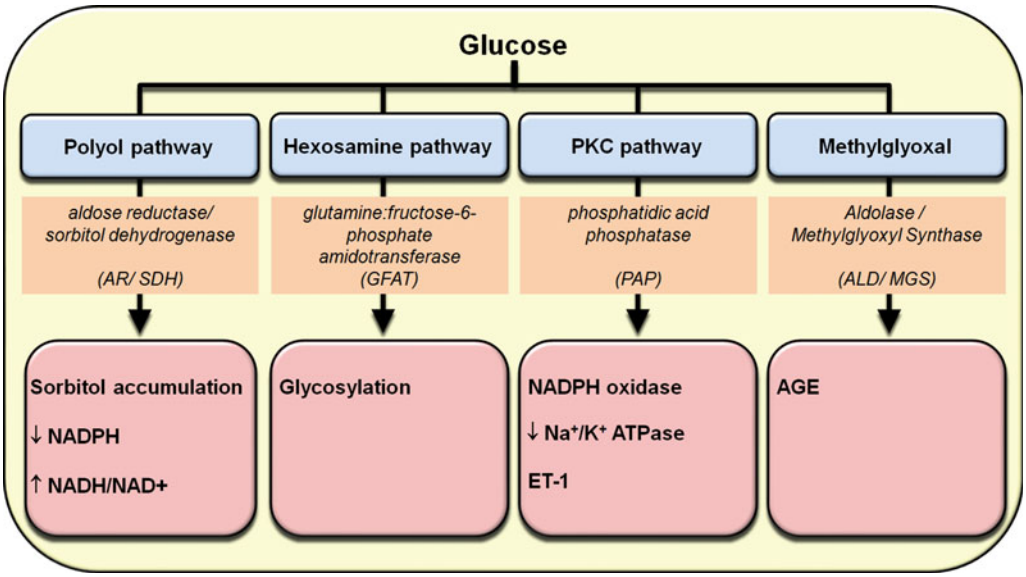

Fig. 3. Early metabolic/biochemical changes in ECs exposed to high levels of glucose. Increased flux of cytosolic glucose through the polyol, hexosamine, protein kinase C, and methylglyoxal pathways represents early alteration in the ECs. Activation of these pathways paves the path for EC dysfunction and loss through elaboration of reactive oxygen species (ROS), loss of vasoregulatory function (endothelin/nitric oxide imbalance), and modification of proteins. Key enzymes involved in these pathways are also indicated. *AGE* advanced glycation end products; *ET* endothelin; *PKC* protein kinase C; *NAD+* nicotinamide adenine dinucleotide; *NADH* nicotinamide adenine dinucleotide, reduced; *NADPH* nicotinamide adenine dinucleotide phosphate, reduced.

Clinical studies show that polymorphisms in AR gene may be linked to increased susceptibility of microvascular complications [82–84]. Although inhibition of AR has not provided any conclusive results, one recent trial with the AR inhibitor sorbinil showed slower rate of microaneurysms in the retina [85]. A new class of AR inhibitors was recently tested in streptozotocin-induced diabetic rats [86], but whether this selective AR inhibitor (ARI-809) produces favorable results in clinical trials remains to be determined.

Hexosamine Pathway

Metabolites of the glycolytic pathway may also be shunted through the hexosamine pathway in diabetes [87]. This pathway produces uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), substrate for *O*-linked glycosylation of serine/threonine-containing proteins and proteoglycan synthesis. Studies have shown that inhibition of the key enzyme in this pathway, glutamine:fructose 6-phosphate amidotransferase (GFAT), reduces hyperglycemia-induced fibrogenic protein expression in aortic ECs [88]. In addition, a large number of proteins that are implicated in the development of diabetic complications are modified by *O*-linked glycosylation. These include protein kinases, growth factors, and transcription factors [89].

Protein Kinase C Pathway

A number of protein kinase pathways are activated when ECs are exposed to high levels of glucose [62, 90–92]. Several studies have shown activation of protein kinase C (PKC) in diabetes [62, 90, 93–95]. There are a number of PKC isoforms that are activated in animal models of diabetes including PKCα, βI, βII, γ, and δ [96, 97]. PKCβI and II show the most prominent level of induction in the retina [97]. We and others have previously shown that PKC may mediate glucose-induced EC permeability [62, 98] and ECM protein production [90]. PKC activation in ECs also causes increased expression of endothelin-converting enzyme-1 and ET-1 [99, 100]. In addition, PKC may also be involved in pericyte loss and expression of various growth factors and vasoactive factors [94, 95, 98, 101, 102]. Several experimental and clinical studies have been carried out with selective PKCβ inhibitor, ruboxistaurin mesylate (LY333531) [103–107]. In phase III clinical trials, ruboxistaurin showed a delay in the occurrence of moderate visual loss in patients with early DR (nonproliferative phase) at 24 months [108].

Activation of Other Protein Kinases

Mitogen-Activated Protein Kinase (MAPK)

Recently, studies have reported an important role of mitogen-activated protein kinase (MAPK) pathway in the diabetic complications [109, 110]. The MAPK family consists of extracellular signal-regulated kinase (ERK) and stress-activated components, namely c-jun N-terminal kinase (JNK) and p38 [110, 111]. We have shown that glucose-induced ECM protein synthesis in cultured ECs is mediated by the activation of the MAPK pathway [90]. We have further demonstrated that MAPK activity leads to activation of transcription factors, nuclear factor-κB (NF-κB), and activating protein-1 (AP-1) [90]. Inhibition of either MAPK or PKC is able to normalize the effects of high levels of glucose. Furthermore, inhibiting PKC in cells exposed to high glucose reduces MAPK activation suggesting an important cross-regulation between PKC and MAPK pathways. It is possible that MAPK activation may also occur in vascular ECs via a PKC-independent pathway [112]. Oxidative stress may cause MAPK activation by ERK5 (big MAPK1/BMK1) [113]. Knocking out BMK1 results in angiogenic defect and embryonic lethality [114]. BMK1, however, differs from other MAPK as it contains a transcriptional activation domain, mediating protein–protein interaction with several other factors [114, 115]. Whether such pathways are also activated in DR remains to be determined.

Protein Kinase B and Serum- and Glucocorticoid-Regulated Kinase (SGK-1)

Cultured ECs challenged with high levels of glucose also show an important role of protein kinase B (PKB) [92] and serum- and glucocorticoid-regulated kinase-1 (SGK-1) [91]. Several growth factors stimulate the activation of PKB. There are three major PKB isoforms α, β, γ. These isoforms belong to a subfamily of protein kinases named AGC protein kinases and include PKC and PKA. PKB can regulate the function of cytoplasmic as well as nuclear proteins [116, 117]. We have shown rapid glucose-induced activation of PKB [92] and SGK-1 [91]. Inhibiting PKB and SGK-1 either by dominant negative transfections and/or small interfering RNA causes complete normalization of

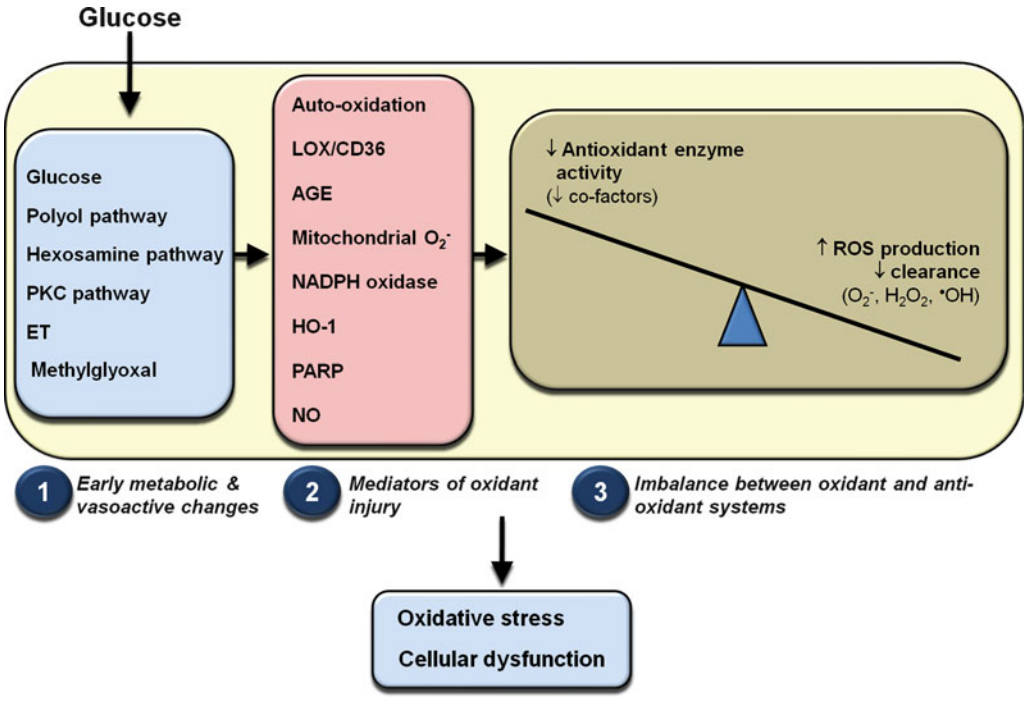

Fig. 4. Mechanisms causing hyperglycemia-induced oxidative stress. High glucose levels directly increase ROS production by autoxidation. Increased flux through the polyol, hexosamine, PKC, and methylglyoxal pathways may also lead to increased oxidative stress. In addition, hyperglycemia may increase ROS indirectly by increasing the activity of various enzymes that lead to oxidative stress. *AGE* advanced glycation end products; *ET* endothelin; *HO* heme oxygenase; *PKC* protein kinase C; *LOX* lectin-like oxidized LDL receptor; *NO* nitric oxide; *PARP* poly(ADP-ribose) polymerase; *SOD* superoxide dismutase.

high glucose-induced FN expression in the vascular ECs. Interestingly, this role of PKB in ECM protein expression is also regulated by both MAPK and PKC [92]. We have further shown that PKB phosphorylation can lead to the activation of NF-κB and AP-1 [92]. These studies suggest that multiple pathways converge on NF-κB and AP-1 to mediate increased ECM protein synthesis.

Increased Oxidative Stress

Increased glucose-induced oxidative stress is another early event in the ECs. There are multiple pathways that increase oxidative stress (Fig. 4). Acute exposure of vascular cells to high ambient glucose causes glucose autoxidation [87] and mitochondrial superoxide production [118–120]. Inhibiting mitochondrial superoxide production has been shown to be beneficial for DR by blocking major pathogenetic pathways [118]. Oxidative stress in diabetes may also be induced by indirect means, which include the NADPH oxidase enzyme [121, 122]. NADPH oxidase may increase superoxide production and through induction of xanthine oxidase. This pathway may

also inhibit superoxide dismutase. Impairment of antioxidant enzymes could also be carried out by increased AR activity through the imbalance between NADP+ and NADPH. A number of other enzymes have also emerged as being important mediators of increased oxidative stress. Lipoxygenase enzyme (LOX) may contribute to diabetes-induced oxidative stress [123]. LOX increases the oxidation of low density lipoproteins (ox-LDLs) [124, 125]. We have shown that glucose increases CD36 (an ox-LDL receptor) and leads to increased uptake of ox-LDL and oxidative DNA damage in vascular ECs [124]. Exposure of pericytes to ox-LDL has also been reported to cause cellular apoptosis [126]. Whether the mechanism involves CD36 in pericytes remains to be determined.

Recently, several investigators have shown a role of poly(ADP-ribose) polymerase (PARP) in cultured ECs and retina of diabetic animals [127–129]. Increased PARP activity, possibly in response to oxidative DNA damage, may cause vascular EC dysfunction by depleting NAD+ and ATP. PARP may also cause NF-κB activation [130]. In a nondiabetic system, PARP activation has been linked to histone deacetylases (HDACs) and transcription coactivator p300 [131, 132]. Whether a similar pathway may also be involved in DR requires further investigation.

Protein Glycation

Accelerated glycation of proteins is also an important mechanism leading to cellular dysfunction in diabetes. High levels of glucose may cause nonenzymatic glycation of both intracellular and extracellular proteins [133, 134]. These modified proteins are recognized by AGE receptors (RAGEs) and possibly other scavenger receptors. Studies have shown that retinal vascular tissue and cultured ECs express both RAGEs and CD36 (a scavenger receptor) [135–139]. Although the mechanisms of AGE-mediated cellular dysfunction are currently being elucidated [140–142], aberrant modification of proteins is expected to alter the function of the proteins. In the case of extracellular proteins, glycation may also lead to aberrant outside-in signaling. Evidence for this comes from studies that show that injecting exogenous AGEs in diabetic animals causes retinal pericyte loss [143]. Interestingly, when retinal ECs are exposed to glycated BM proteins [144], the cells proliferate. A specific inhibitor of nonenzymatic glycation, aminoguanidine, has been shown to prevent retinal microaneurysms, acellular capillaries, and pericyte loss in the diabetic dogs [145]. In clinical trials, however, modest beneficial effects were noted [146].

Aberrant Expression of Growth Factors

A number of growth factors have been implicated in the pathogenesis of DR. Growth factor alterations are believed to mediate BM thickening, EC hyperplasia, and unregulated angiogenesis [147]. The list of growth factors that exhibit altered expression in vitreous of diabetic patients or retinal tissues of diabetic rats is a long one [147]. Important growth factors include insulin-like growth factor-1 [148], platelet-derived growth factor [149], basic fibroblast growth factor [150], transforming growth factor-β [151], and VEGF [152]. These growth factors have been shown to induce EC proliferation, ECM synthesis (especially in the case of TGF-β), and cause retinopathy-like lesions in animals [147].

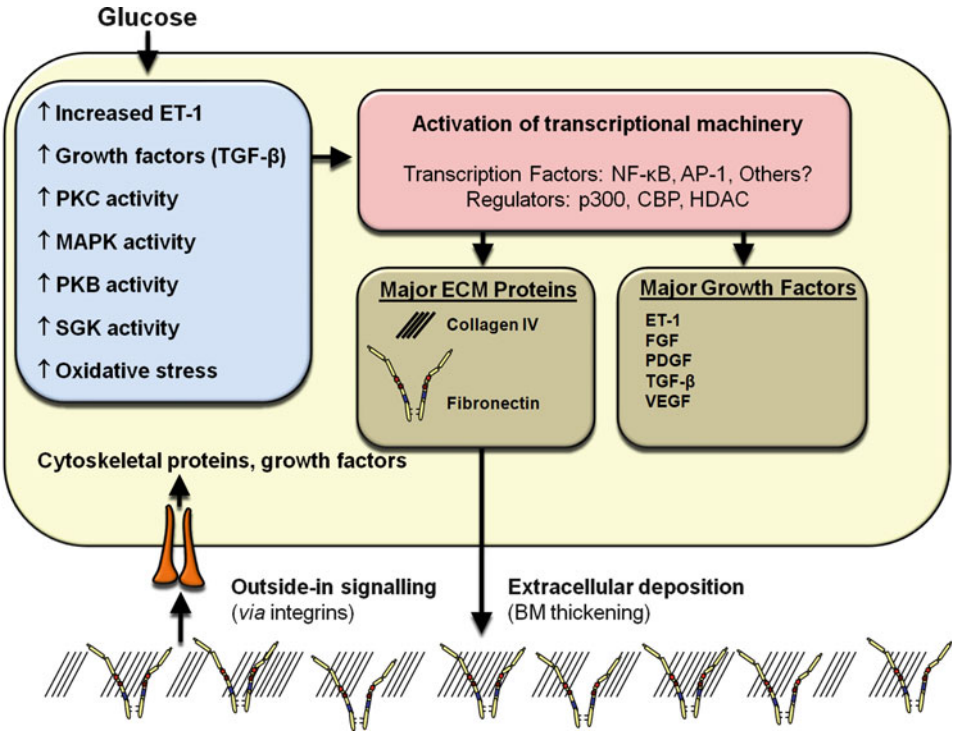

Fig. 5. Mechanisms of glucose-induced growth factor and ECM protein expression in ECs. High levels of glucose lead to activation of a number of intracellular signaling proteins. These signaling proteins mediate the effects of glucose by activating transcription factors and altering other transcriptional regulators (coactivators/corepressors). Transcription factor activity then leads to increased expression of key ECM proteins and growth factors. *AP-1* activating protein-1; *BM* basement membrane; *ET* endothelin; *FGF* fibroblast growth factor; *MAPK* mitogen-activated protein kinase; *NF-kB* nuclear factor-κB; *PKB* protein kinase B; *PKC* protein kinase C; *PDGF* platelet-derived growth factor; *SGK* serum- and glucocorticoid-regulated kinase; *VEGF* vascular endothelial growth factor.

Transcription Factors

All glucose-induced signals converge on transcription factors to regulate expression of key genes involved in vascular function (Fig. 5). Two main transcription factors with wide range of activities are NF-κB and AP-1. NF-κB is a redox-sensitive transcription factor. In quiescent cells, NF-κB exists as an inactive dimer bound to an inhibitory protein, IκB. Upon stimulation, IκB is degraded and NF-κB translocates to the nucleus [153]. In diabetes, NF-κB is believed to be activated by a number of factors including ROS and ET-1 [63, 154]. Interestingly, ET-1 expression may also be regulated by NF-κB activity [155]. Studies have reported nuclear NF-κB immunoreactivity (activated state) in the pericytes but not ECs of human diabetic eyes [156]. In experimental diabetes, however, NF-κB activity is evident in retinal vessel ECs [130, 157–159]. Furthermore, cultured ECs show increased NF-κB activity and downstream effects when exposed to high levels of glucose [63, 128, 130, 137, 139, 160]. We have also shown that

ECM protein expression in ECs and retinas of diabetic animals is dependent on NF-κB activity [63, 154].

AP-1 transcription factors [161, 162] are also implicated in ECM protein expression in diabetes. We have shown that high glucose activates MAPK, increases ECM protein expression, and that this pathway is dependent on both NF-κB and AP-1 activation [90]. Triamcinolone acetonide, an inhibitor of both NF-κB and AP-1, has been reported in clinical trials to reduce vascular permeability, hemorrhages, and neovascularization in DR [114, 163, 164]. Several other transcription factors may play regulatory role in these pathways. Most recent studies show that forkhead transcription factors of the O family (FoxO) may also be involved in diabetic vascular dysfunction [165]. FoxOs are ubiquitously expressed including in the brain [166] and have been implicated in cellular proliferation and growth [167]. Exposure of ECs to high glucose increases FoxO1 activation and mediates cellular apoptosis [165]. Diabetic animals, both streptozotocin-induced diabetic rats and Zucker rats, show activation of FoxO1 in the retina which precedes the formation of acellular capillaries. Inhibiting FoxO1 in cultured cells or in diabetic animals reverses cellular dysfunction and apoptosis. Similar to NF-κB, the mechanism of FoxO1 activation involves oxidative stress [165, 168]. Interestingly, FoxO1 may also facilitate eNOS dysfunction and oxidation of LDL [168].

Transcription Regulators

One of the emerging fields in diabetes research is the epigenetic regulation of gene expression. Chromatin structure and access to transcription factors is regulated by a number of modifications including acetylation, methylation, and phosphorylation [169]. One of the extensively studied processes is the acetylation and deacetylation of histone residues. Two main classes of proteins, acting in opposing manner, regulate acetylation and deacetylation. Histone acetyltransferases (HATs) and HDACs control several cellular processes through regulating transcription factors [170]. The best characterized HATs are p300 and CREB-binding protein (CBP) [170]. These HATs add an acetyl group on lysine residues of histones 3 and 4 (H3 and H4). It is believed that addition of acetyl groups leads to chromatin relaxation and access to transcription factors. Involvement of HATs and HDACs in diabetic complications becomes evident when we consider that transcription factors such as NF-κB remain inactive even after nuclear translocation without the association of p300 [170, 171]. We and others have also shown that NF-κB activity in diabetes is regulated by p300 [128, 172]. In addition, FN expression, in both cultured ECs and the retina of diabetic rats, is mediated by p300 induction [128]. Whether HDACs also modulate these pathways is not clear.

Another mode of chromatin remodeling is regulated by enzymes that add or remove a methyl group. Similar to acetylation/deacetylation, methylation/demethylation may also lead to increased or decreased expression of the target genes. Recently, Reddy et al. [173] showed that smooth muscle cells isolated from diabetic animals exhibit increased monocyte chemotactic protein-1 and interleukin expression via methylation of histone-3 lysine-4 (H3K4). Interestingly, this methylation was found near the NF-κB response element. The same group has also shown reduced histone-3 lysine-9 trimethylation at the promoter region of these target genes [174]. A similar phenomenon is also evident in ECs [175, 176]. A brief exposure of aortic ECs to high glucose levels was associated with increased NF-κB p65 expression and H3K4 monomethylation at the NF-κB p65

promoter region [176]. What is fascinating is that these modifications produce long-term phenotypic changes in the cultured cells even following removal of the high glucose stimulus. This has lead to the concept that histone modification may indeed dictate diabetic/metabolic/hyperglycemic memory.

CONCLUDING REMARKS

Diabetes leads to vascular disruption in selected organs that include the retina.

Experimental evidence from animal models and cultured cells suggests that various signaling pathways in concert lead to the pathogenetic changes in the retinal vascular bed. Early adverse effects of high glucose levels may be mediated by metabolic changes (polyol pathway, hexosamine pathway), vasoactive factors (ET and NO), and oxidative stress (leading to EC dysfunction and loss). Aberrations in EC function may then be perpetuated by continued activation of intracellular signaling proteins such as PKC, PKB, MAPK/ERK, and transcriptional regulators (NF-κB and AP-1, p300). Further investigation as to how these signaling pathways interact is timely. Recent evidence of epigenetic changes producing the "diabetic phenotype" supports the notion that a solid understanding of the hyperglycemia-induced transcription machinery is the only means to identifying the molecular signature and point of convergence in DR.

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