Chronic Diabetic Complications: Endothelial Cells at the Frontline

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Abstract: We are approaching the 100-year anniversary of Dr. Frederick Banting's discovery of insulin for the treatment of diabetes. The population of patients with diabetes is ever-increasing. However, with the availability of insulin, the cause of mortality has shifted from diabetic coma and ketoacidosis to chronic secondary complications. Dysfunction and failure of selected organs is now the most prominent cause of morbidity and mortality. These 'target' organs include the eyes, kidneys, heart, lower limbs, and the nervous system. A common feature of these complications is the aberration in the vasculature of the target organs. Studies over the past few decades have shown that dysfunction of the vascular endothelial cells may be the key to the development and progression of the chronic diabetic complications. We present the hypothesis and supporting evidence that preservation and restoration of the endothelial cell function may potentially be the most efficient means of targeting the adverse effects of diabetes.

Keywords: Endothelial cells, diabetes, microangiopathy, macroangiopathy, basement membrane, extracellular matrix, fibronectin, oxidative stress, protein kinase c, aldose reductase, nitric oxide, endothelin, vasoactive factors, cardiomyopathy, advanced glycosylation end products, metabolic distress, glucose.

INTRODUCTION

According to the most recent estimates, diabetes affects over 6% of the world and 9% of the North American population[1]. This equates to an enormous economic burden[1]. Because of our lifestyle and yet to be untangled genetic determinants, a possible plateau or a decrease in this upward trend is nowhere in sight. The use of insulin has significantly lowered the high rate of mortality due to diabetic coma and ketoacidosis. Unfortunately, we have also witnessed a shift in the cause the diabetes-associated morbidity and mortality due to chronic secondary complications. These complications target selected organs and

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manifest as angiopathy involving both the micro- (retinopathy, nephropathy, neuropathy, and cardiomyopathy) and macro- (accelerated atherosclerosis) vascular systems[2,3]. Macroangiopathic changes may also affect the coronary artery, the carotid artery, as well as peripheral arteries. Identification of the underlying cause of these secondary complications is warranted and ever so timely. The first indication as to the potential cause comes from clinical studies conducted in the 1990s[4,5]. The seminal findings of these studies pointed to hyperglycemia as the key mediator of target organ dysfunction in diabetes[4,5]. There may be other factors that potentiate and contribute to the adverse effects of hyperglycemia such as hyperlipidemia and hyperinsulinemia. However, if we can achieve a tight glucose control, which is a daunting task in itself, we can prevent the development and progression of the complications[4,5]. This chapter will explore the hypothesis that vascular endothelial cell dysfunction is the origin of chronic diabetic complications and may very well be the most important therapeutic target.

ENDOTHELIAL CELLS – THE PRIMARY TARGET OF HYPERGLYCEMIC DAMAGE

Endothelial cells (ECs) line the blood vessels and are critical for the function and integrity of the vascular unit. ECs were first considered to be merely a non-thrombogenic cellular barrier. However, Florey and colleagues (1966) suggested that EC lining is dynamic, metabolically relevant for tissue function, and represents more than an inert cell sheet [6]. Soon after, the first successful *in vitro* EC culture was established[7]. This paved the path to the greater understanding of the role of ECs in the vascular system. We can now appreciate the important role ECs play in regulating vascular tone (blood flow and pressure), permeability, blood fluidity, thrombotic/fibrinolytic balance, and leukocyte traffic[8].

Due to the anatomical location and barrier function, ECs are the first cells to encounter circulating glucose. ECs incorporate glucose via facilitative diffusion[9,10] and changes in the level of glucose does not lead to altered expression of the primary EC glucose transporter, glucose transporter-1 (Glut1)[9,10]. This is in contrast to the contractile cells (smooth muscle cells and pericytes) in which Glut1 levels decrease following exposure to high levels of glucose[9,10]. Therefore, increased or decreased plasma glucose levels may have profound effects in the ECs. Under physiological conditions (glucose levels ~ 5 mM or 90 mg/dL), glucose is primarily metabolized by the glycolytic pathway with the hexose monophosphate pathway and Krebs cycle accounting for less than 2% combined[11]. However, the oxidation of glucose through the Krebs cycle can be increased when glucose levels drop below 1 mM (18 mg/dL)[11]. The same holds true when glucose reaches supraphysiological levels[12]. In addition, higher than physiological levels of glucose also activate alternative metabolic pathways such as the polyol and hexoseamine pathways (Fig. **1**)[12,13]. High levels of glucose cause biochemical changes in the ECs occur which are reminiscent of early molecular alterations in the target organs of diabetes[14-16]. *In vitro* studies have shown that exposure of EC to high glucose levels leads to activation of the EC which manifests as increased production of the extracellular matrix proteins (major proteins being collagen and fibronectin), procoagulant proteins (von Willebrand Factor; vWF), and altered cellular activities (proliferation and migration)[17-22]. Other factors, such as increased insulin levels, may also contribute to these factors[23-25]. Thus, all of the normal EC functional properties (summarized in Table **1**) may be affected by glucose and/or insulin in diabetes, making EC dysfunction a key pathological element in the development of chronic diabetic complications [26,27].

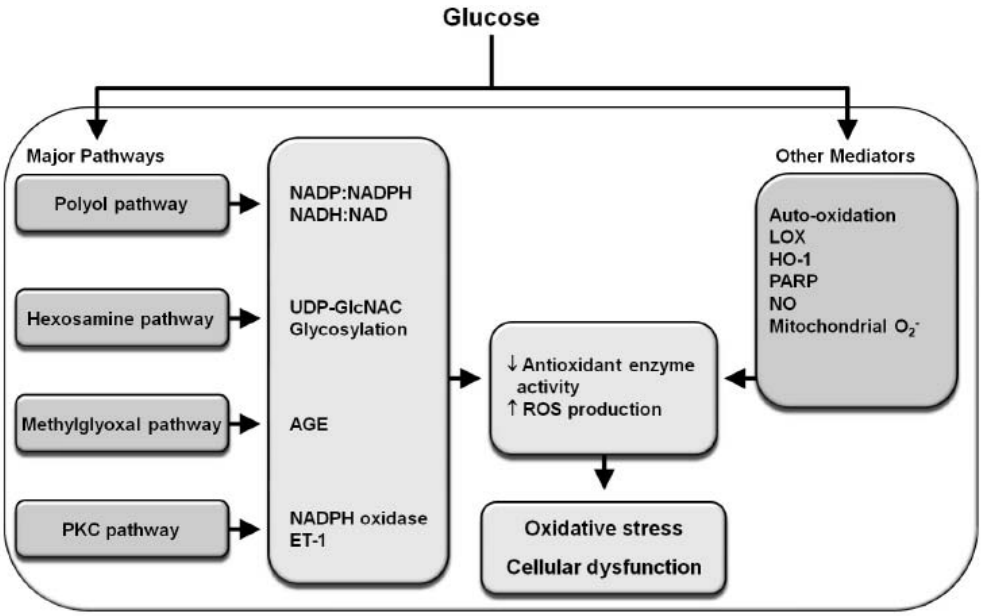

Fig. (1). Proposed mechanisms of glucose-induced biochemical changes in the ECs. High glucose levels lead to activation of polyol pathway, hexoseamine pathway, protein kinase C, AGE formation, and PARP activation. In addition, glucose may increase oxidative stress indirectly by activating lipoxygenase and heme oxygenase enzyme systems. These biochemical changes may culminate in increased ET-1 activity, reduced NO availability, oxidative stress and EC dysfunction [AGE = advanced glycation end product; ET-1 = endothelin-1; HOE-1 = heme oxygenase-1; LOX = lipoxygenase; NAD+ = nicotinamide adenine dinucleotide; NADH = nicotinamide adenine sinucleotide, reduced; NADP = nicotinamide adenine dinucleotide phosphate; NADPH = nicotinamide adenine dinucleotide phosphate, reduced; NO = nitric oxide; PARP = poly(ADP-ribose) polymerases; PKC = protein kinase C; ROS = reactive oxygen species; UDP-GlcNAC = UDP-N-acetylglucosamine].

Subsequent to EC dysfunction, the target tissues in diabetes exhibit poor blood flow and ischemia (Fig. **2**)[15,16,28]. This is brought upon by the combined effect of functional and structural alterations in the vasculature. The response of the target organs to reduced blood flow and ischemia, however, varies depending on the vascular bed. The retina (and possibly the kidney) responds to EC dysfunction and ischemia by enhancing new blood vessel formation[28]. Other target organs exhibit impaired vessel formation[29]. This suggests that the EC dysfunction may not be uniform in all organs in the patient with diabetes. The mechanism by which chronic diabetes leads to impairment of blood supply in some target organs while inducing unregulated angiogenesis in others remains undetermined. Selectivity in the target organs may suggest the importance of two key elements, the tissue microenvironment and the intrinsic properties of the targeted ECs. It is well known that ECs in the vasculature are heterogeneous[8] (as a product of both intrinsic and extrinsic properties). The variability in the ECs is evident at the level of cell size, cell shape, antigenicity, function, and susceptibility to pathological states. For example, Weible-Palade bodies (site of vWF storage in ECs) exhibit differential patterning in the vasculature being highest in number in the ECs close the heart as compared to other vascular beds[30-32]. The

Table 1. EC Function – and their Mediators Altered in Diabetes

EC Function	Mediators
Hemodynamic/Vascular Tone	*Vasoconstriction* Angiotensin II Endothelin-1 Thromboxane A_2 Prostaglandins H_2 and $F_{2\alpha}$ *Vasodilators* Bradykinin Nitrix Oxide Prostaglandin I_2
EC Growth and Proliferation	*Proliferation of ECs* basic-Fibroblast Growth Factor Endothelin-1 Epidermal Growth Factor Hepatocyte Growth Factor Insulin-like Growth Factor (1 and 2) Nerve Growth Factor Oncofetal Fibronectin Platelet Derived Growth Factor Transforming Growth Factor-β Tumor Necrosis Factor-α Vascular Endothelial Growth Factor *Inhibition of EC proliferation* Angiostatin Endostatin Nitric Oxide Pigment Epithelium Derived Factor Prostaglandin I_2
Anti-thrombogenic/ -fibrinolytic	*Pro-thrombogenic/fibrinolytic* Plasminogen Activator Inhibitor-1 von Willebrand Factor *Anti-thrombogenic/fibrinolytic* Nitric Oxide Tissue type-Plasminogen Activator Thrombomodulin
Inflammation	*Pro-inflammatory* E-selectin Intracellular Adhesion Molecules-1 Vascular Adhesion Molecule-1 *Anti-inflammatory* Nitric Oxide

microenvironment may also change the EC phenotype. When aortic ECs are cultured on lung-derived extracellular matrix substrate, the cells begin to express lung endothelial cell adhesion molecule-1 (Lu-ECAM-1)[33], a marker specific to lung endothelium. The same is true for other organ extracellular matrix proteins. Culturing of bovine adrenal cortex ECs on kidney cell-derived matrix has been shown to cause fenestrae formation in the ECs[34].

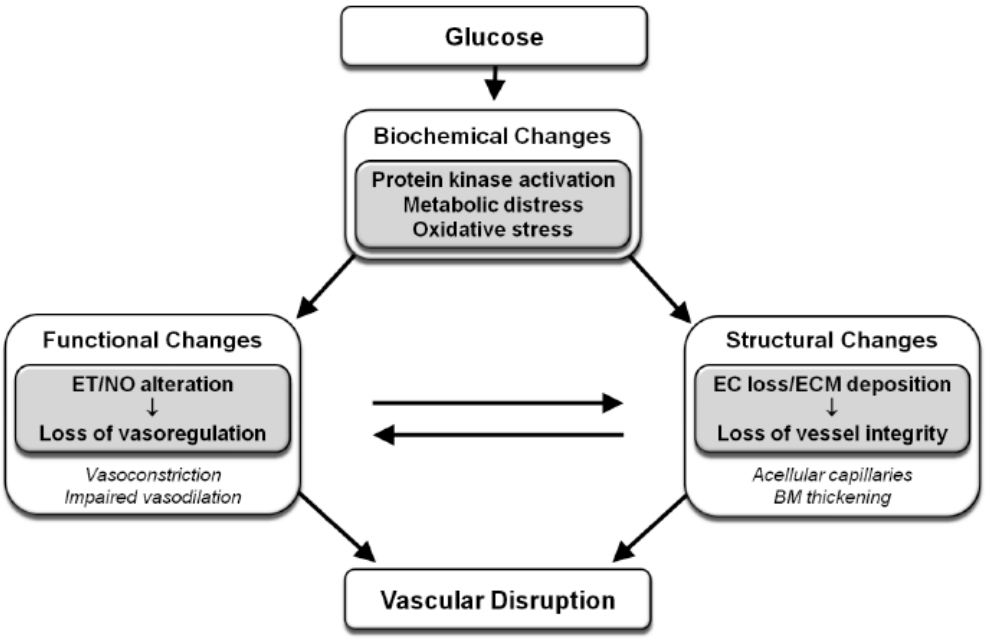

Fig. (2). Proposed mechanisms leading to vascular disruption in diabetes. High circulating glucose levels lead to biochemical dysfunction in the target organ vascular ECs. These biochemical changes include protein kinase activation, metabolic changes, and increased oxidative stress. As a result, both structural and functional aspects of the blood vessels are altered. Impaired vasoregulation, increased permeability, extracellular matrix expansion, and dysfunction of the vascular cells causes reduced blood flow in the target organ [BM = basement membrane; EC = endothelial cell; ECM = extracellular matrix; ET = endothelin; NO = nitric oxide].

Numerous biochemical and molecular changes within the vascular ECs lead to functional and structural alterations of the target organ vascular bed. There are three distinct events, analogous to a 3-act play that orchestrates the complex pathology of the vasculature in diabetes. These include a) damage, b) response, and c) remembering the unforgettable.

ACT 1: ALTERED VASOACTIVE FACTORS & REDUCED BLOOD FLOW IN TARGET ORGANS

EC dysfunction in the microvascular complications of diabetes is well established[16]. Even macrovascular atherogenesis exhibits an important EC component [35,36]. ECs are important for the initiation and perpetuation of atherosclerotic process[37-39]. The initial events may include increased antiginicity and permeability whereas the later events may encompass the retention of atherogenic proteins as well as leukocytes. EC dysfunction has

traditionally been defined as an impairment of endothelium-dependent vasodilation (EDV) in diabetes[40-42]. This is one of the earliest functional changes and it has been shown to precede the structural changes in the vasculature[43-48]. Studies have reported that acute exposure to high levels of glucose in healthy individuals results in impaired EDV[49,50]. The underlying mechanism follows a two-hit model: an increase in expression/activity of vasoconstrictors and a concurrent decrease in expression/activity of vasodilators. The major vasoactive factor that is altered in diabetes is endothelin-1 (ET-1). ET-1, the most potent endogenous vasoconstrictor, is a short peptide that is primarily produced by the ECs[51,52]. Dysfunction and/or activation of the ECs is, therefore, expected to alter the levels of ET-1 production. Augmented ET-1 levels have been reported in diabetes and have been shown to mediate vasoconstriction and reduced blood flow[14]. Increased activity of ETs has also been shown in the resistance arteries of diabetic patients[53]. Moreover, administration of exogenous ET-1 in humans reduces coronary and renal blood flow and increases vasoconstriction[54-56]. This suggests that inhibiting ET-1 may alleviate impaired EDV in diabetes. Improvement of the vasodilator responses have been noted in diabetic patients administered an ET antagonist (selective ETA receptor blocker BQ-123)[53]. ETA receptors are the main receptors present on the perivascular cells that mediate ET-induced vasoconstriction[14]. We have also reported that diabetes-induced retinal capillary vasoconstriction in experimental diabetes is normalized with a dual ET receptor antagonist (Bosentan/ dual ETA and ETB receptor blocker)[57].

As mentioned above, a concurrent alteration in the vasodilators may be required for EDV impairment in diabetes. Acetylcholine-induced paradoxical vasoconstriction in atherosclerotic coronary arteries have suggested an important role of nitric oxide synthase (NOS) enzymes[58]. NOS are a family of enzymes that produce the vasodilator, nitric oxide (NO). There are three members of the family: neuronal NOS (type I), inducible NOS (type II), and endothelial NOS (type III). Acute exposure of ECs to glucose decreases NO generation by agonists such as bradykinin and A23187[59]. Purified endothelial NOS when assayed in the presence of glucose also shows significantly lower level of NO production[59]. In addition to reduced generation, NO may also be sequestered by glucose-induced oxidative stress[14-16]. This creates an imbalance in the counter-activity of ETs and NO. Therefore, similar to the approach of inhibiting ET-1, we could potentially increase NO production to normalize EDV impairment. In support of this as a potential therapeutic approach, we have previously shown that treatment of diabetic animals with a NO donor, molsidomine, prevents diabetes-induced vasoconstriction in the retina [60]. Recent studies have also documented restoration of EC function by overexpression of endothelial NOS [61,62].

The mechanism of vasoactive factor alteration and scavenging is intimately related to increased oxidative stress. Increased glucose-induced oxidative stress is an early change in the ECs. The pathways which increase oxidative stress are multifactorial (Fig. **1**). Acute exposure to high ambient glucose causes glucose auto-oxidation [13]. A number of other pathways also induce oxidative stress including, protein kinase C activation, polyol and hexoseamine pathways, oxidized-low lipoprotein, and heme oxygenase pathway [63]. Oxidative stress, together with vasoactive factors, represents the major avenues that can be targeted to restore EC function (Table **2**). In fact, a number of patented dugs are available to achieve exactly that (Table **3**).

Table 2: Possible Pharmacological Targets for Restoration of EC Dysfunction in Diabetes

Targets	Effect
Vasoactive Factors	Vascular tone, cellular proliferation, ECM deposition
Angiotensin II	Vascular tone, growth factor expression
ET-1	
NO	
Oxidative Stress	
AGE/RAGE	
Aldose Reductase	
Heme Oxygenase	
Nuclear Factor-κB	
NADPH Oxidase	
PARP	
Protein Kinase C	
Others*	Cellular proliferation
ED-B$^+$ Fibronectin	
MAPK	
Protein Kinase B	
SGK-1	
VEGF	

*Increase/decrease in cardiomyopathy/retinopathy. [AGE = advanced glycation end product; ED-B$^+$ FN = Extra domain-B containing fibronectin; ET-1 = endothelin-1; MAPK = mitogen activated protein kinase; NADPH oxidase = nicotinamide adenine dinucleotide phosphate-oxidase; NO = nitric oxide; PARP = poly (ADP-ribose) polymerase; RAGE = receptor for AGE; SGK-1 = serum- and gluococrticoid-regulated kinase-1; VEGF = vascular endothelial growth factor]

Table 3. Selective Agents for the Restoration of EC Dysfunction in Diabetes

Parameter/Targets	Agents	Patents [Reference]
Vascular Tone	Enalapril	US 4374829 [115]
ACE Inhibitors	Lisinopril	US 4374829 [115]
ET Antagonists	Quinapril	US 4344949 [116]
NO Prodrugs	Ramipril	US 4587258 [117]
	Perindopril	US 4508729 [118]
	Fosinoprol	US 4337201 [119]
	benazapril	US 4410520 [120]
	A127722	US 6573285 [121]
	ABT 627	US 20050042172 [122]
	Bosentan	US 20030176356 [123]
	BQ123	US 6573285 [121]
	BQ 610	US 20030176356 [123]
	BQ788	US 20030176356 [123]
	FR 139317	US 20030176356 [123]
	LU 135252	US 20030176356 [123]

Parameter/Targets	Agents	Patents [Reference]
	PD 142893	US 20030176356 [123]
	PD 14565	US 20030176356 [123]
	PD 156707	US 6573285 [121]
	RES 701-1	US 6855701 [124]
	SB 209670	US 6573285 [121]
	TAK 044	US 6573285 [121]
	AcOM-DEA/NO	US 5366997 [125]
	DETA/NO (NOC-18)	US 5155137 [126]
	DPTA/NO (NOC-19)	US 5155137 [126]
	MAHMA/NO (NOC-9)	US 5155137 [126]
	PAPA/NO (NOC-15)	US 5155137 [126]
	PROLI/NO	US 6379660 [127]
	SPER/NO	US 5155137 [126]
	V-PYRRO/NO	US 5366997 [125]
Oxidative Stress	Benfotiamine	US 3064000 [128]
AGE Blockers/Breakers	ALT-711	US 6790859 [129]
ARI	Thiazolium compounds	US 6849629 [130]
PKCβ Inhibitor	Pyridazinone	To be filed
NADPH Oxidase	Arxxant	US 5902831 [131]
PARP	Apocynin	US 5990137 [132]
	Diapocynin	WO 9307868A1 [133]
	3-AB	WO 9307868A1 [133]
	NU 1025	WO 9911624A1 [134]
	6-(5H)-phenanthridinones	WO 9911624A1 [134]
	4-iodo-3-nitrobenzamide	WO 9911645A1 [135]
	GPI-6150	WO 991649A2 [136]
	DPQ	WO 9704771A1 [137]
	Benzimidazole-4-carboxamides	WO 0026192A1 [138]
	2-aryl-1H-benzimidazole-4-carboxamides	WO 0236576A1 [139]
	4-aryl-phthalazones	WO 0142219A2 [140]
	PJ34	

[ACE = angiotensin-converting enzyme; AGE = advanced glycation end product; ARI = aldose reductase inhibitors; ET-1 = endothelin-1; NADPH oxidase = nicotinamide adenine dinucleotide phosphate-oxidase; NO = nitric oxide; PARP = poly (ADP-ribose) polymerase; PKCβ = protein kinase C isoform β].

ACT 2: GROWTH FACTORS AND MATRIX PROTEINS REGULATE THE BALANCE BETWEEN FIBROSIS & NEOVASCULARIZATION

We have recently proposed that a balance exists between fibrosis and neovascularization in diabetic complications [16]. The balance is shifted towards EC proliferation and neovascularization in diabetic retinopathy (and possible nephropathy) and towards scar formation/ fibrosis in diabetic cardiomyopathy and neuropathy. This balance was recently highlighted in diabetic retinopathy [64]. Understanding the differences between such contrasting effects

of diabetes on the target organs is critical. Tissue microenvironment, intrinsic EC differences, as well as presence of other risk factors (hyperlipidemia, hyperinsulinemia) may be involved in the process. Current molecular understanding of the changes in the target organs shows the involvement of the same players such as metabolic distress, oxidative stress, and protein kinase C.

In retinopathy, the oxygenation state regulates various growth factors that promote angiogenesis in order to counter the effects of reduced blood flow and non-perfusion from altered vasoactive factors and cellular loss [65]. However, it is believed that unregulated expression of these growth factors leads to uncontrolled retinal neovascularization and blindness in the diabetic patients. These growth factors may also initiate elaboration of the mediators that are not necessarily regulated by tissue oxygenation. Similar to the retina, structural changes in the kidney in diabetes may also include increased angiogenesis. An increased glomerular filtration rate which may, in part, be due to an increased surface area has been reported in experimental diabetes[66]. This study has shown a 30-50% in surface area per glomerulus and 20-30% increase in the number of capillaries in that diabetic rat kidneys[66]. However, in contrast to the retina [16,28] and possible kidney [66], diabetes leads to impairment in the neovascularization of the heart. It has been reported that diabetes causes reduced expression of vascular endothelial growth factor (VEGF) and its receptors in the myocardium [67]. VEGF is an EC-specific mitogen and has been demonstrated to cause angiogenesis in a number of disease models. The reduced VEGF expression in the heart is in contrast to the retina where high levels of VEGF have been shown[28]. In addition, collateral vessel formation has also been reported to be impaired in the hearts in diabetes [68-70]. One possible explanation could underlie defective VEGF signaling in monocytes which are important for the process of arteriogenesis [69]. Activated monocytes have been shown in ischemic tissues [71,72]. Monocytes migrate in response to VEGF through the activation of VEGFR1 (also known as Flt-1). *In vitro* assays have shown that monocytes isolated from diabetic patients fail to migrate in response to VEGF [73]. Taken together, these findings suggest that reduced expression of growth factors and growth factor receptors and impaired signaling may in concert lead to inadequate neovascularization in the myocardium. Further studies investigating the contribution of 'angiogenesis' and 'arteriogenesis' in the heart are required before diabetic cardiomypathy may be adequately targeted.

Another mechanism of differential effects of high glucose levels could be the change in the microenvironment of the vascular cells. The vascular unit comprises two major cellular components (ECs and the perivascular cells) and the surrounding scaffolding proteins, extracellular matrix (ECM). The role of ECM in EC biology spans from proliferation, migration, and stabilization. Binding of the EC surface integrins to the ECM regulates cell survival/apoptosis, growth, as well as cytoskeletal changes [74]. The heterogeneity in the ECM, therefore, is important in EC function. Growth factor-induced changes in the ECs are also matrix-dependent [75]. Moreover, the neutralizing effect of angiostatic proteins (thrombospondin, endostatin, etc.) on growth factor-induced proliferation and migration also depends on the ECM [76].

ECM changes during tumorigenesis, which is believed to promote neovascularization, include increased expression of fibronectin (FN), collagen, and tenascin-C [77-79]. Whereas, laminin and collagen (IV) levels are decreased. These selective ECM protein changes are thought to invoke a proliferative response in the vascular ECs during angiogenesis [75]. A similar scenario is evident in the retinal vascular development [77]. In

the retina, FN has been reported to be expressed in the active zones of vascularization [77]. Moreover, pericytes and laminin in the basement membranes (BM) appear with EC differentiation and vessel maturation. Could differential temporal/spatial expression of FN and laminin dictate increased angiogenesis in the retina while causing impaired vessel formation in the heart? Retinal BMs of diabetic animals have been shown to contain increased collagen α1 (IV), β1 and γ chains of laminin, and FN [80]. These changes are brought upon as early as 8 weeks following onset of diabetes [80]. In addition, upregulation of tenascin has been reported in retinal vessels of diabetic patients and animals [81,82]. Other BMs in diabetic patients exhibit somewhat similar ECM protein profile [83-86]. In the rat myocardium, diabetes increases laminin expression at 2 months following onset [87]. Two simplistic theories can be formulated to explain these findings, a) proliferating ECM proteins outweigh differentiating ECM proteins, and b) differential proteolytic processing of ECM proteins may produce fragments which confer an 'angiogenic' phenotype in the ECs. In support of such a notion, we have recently shown increased expression of an embryonic splice variant of FN (ED-B^+ FN) in the vitreous samples of proliferative diabetic retinopathy [88]. Targeted inhibition of ED-B^+ FN is associated with decreased EC proliferation and tube morphogenesis [89]. It is also plausible that FN, which undergoes extensive proteolytic processing [90], produces angiogenic fragments [91,92].

Lastly, in terms of the microenvironment, the presence of abundant cardiac fibroblasts may contribute to impaired vessel formation. In the heart, cardiomyocytes constitute about 70% of the volume but only 30% in terms of cell number [93,94]. ECs outnumber the cardiomyocytes by a ratio of 3:1[93,94]. A significant number of cardiac fibroblasts are also present in the heart which may be responsible for the cardiac ECM deposition in addition to the cardiac ECs. Angiotensin II is able to cause cardiac fibroblast proliferation and collagen deposition [95,96]. Studies have also indicated that angiotensin II exerts the action on cardiac fibroblasts through the induction of ET-1 [97,98]. In addition to ECs, fibroblasts also express ET receptors [99]. *In vitro* studies reveal that isolated rat fibroblasts increase production of ECM components upon ET administration [99-101]. These changes can be readily blocked by ET antagonists. It has further been shown that endocardial capillary ECs (in culture) show a 25% higher growth index as compared to other vascular ECs. These findings indicate that tissue micrenvironment is an important regulator of impaired angiogenic response in the heart which could be mediated by increased ECM deposition. In clinical trials, ET antagonists have shown promise in short-term studies as indexed by improved cardiac index and pulmonary capillary pressure [102,103]. However, long-term studies have yet to show beneficial effects [104].

An important unresolved question is why do the ECs of new blood vessels (in the retina for example) not susceptible to the glucose-induced damage? Part of the answer may lie in the protein that transports glucose to the ECs. A recent study of the human diabetic retina has shown that Glut1 is expressed in the nerve fiber layer, Muller cells, ganglion and the photoreceptors, the retinal capillaries and the retinal pigment epithelium [105]. Comparing these results to the retina specimens of non-diabetic subjects, it becomes evident that there is no significant change in the pattern or level of Glut1 expression. However, ECs of the neovascular membranes in advanced retinopathy (which entails increased EC proliferation and angiogenesis) did not show expression of Glut1 [105]. This implies that the mechanism by which the new blood vessels escape glucose-induced damage and cellular loss may involve reduced glucose transport and accumulation in the ECs.

ACT 3: THE LASTING MEMORY

A fascinating aspect of molecular changes that mediate the adverse effects of high glucose levels is that the phenotypic changes produced in the vascular cells are long-term. This is dubbed diabetic or hyperglycemia memory [106]. Recent studies attribute this memory to chromatin remodeling. According to this postulate, glucose-induced alteration of enzymes regulating methylation/demethylation causes long-term increases or decreases in target gene expression. Most recently, smooth muscle cells isolated from diabetic animals showed increased monocyte chemotactic protein-1 and interleukin expression which was mediated by methylation of histone-3 lysine-4 near the nuclear factor-κB (NF-κB) response element [107] and reduced histone-3 lysine-9 tri-methylation at the promoter region of these target genes [108]. A similar phenomenon is also seen in the ECs [109,110]. A brief exposure of aortic ECs to high glucose levels was associated with increased NF-κB p65 expression and histone-3 lysine-4 monomethylation at the NF-κB p65 promoter region [110].

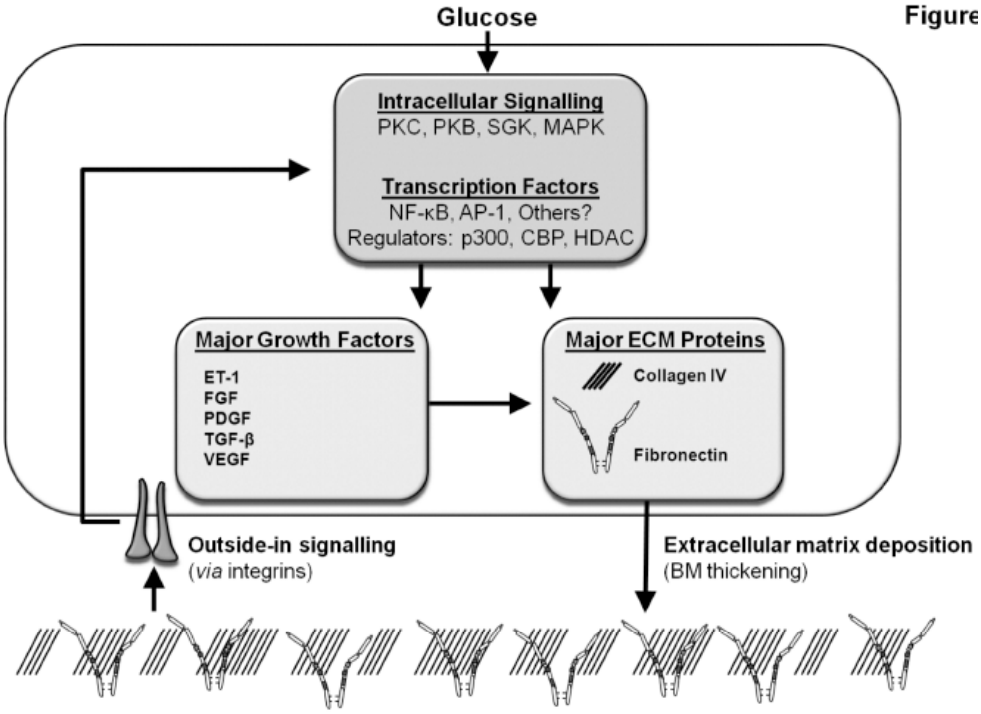

Fig. (3). Effect of glucose on growth factor and ECM protein expression. High levels of glucose increase expression of various growth factors which alter the ECs and cause increased ECM protein expression. Upregulated ECM proteins then signal to the ECs via ECM receptors, integrins. This may represent a feedback loop that mediates continuous adverse effects of hyperglycemia [AP-1 = activating protein-1; BM = basement membrane; bFGF = basic-fibroblast growth factor; CBP = CREB binding protein (CREB = cAMP-response element binding protein); ET-1 = endothelin-1; HDAC = histone deacetylase; MAPK = mitogen-activated protein kinase; NF-κB = nuclear factor-κB; PDGF = platelet-derived growth factor; PKB = protein kinase B; PKC = protein kinase C; SGK = serum- and glucocorticoid- regulated kinase; TGF-β = transforming growth factor-β; VEGF = vascular endothelial growth factor].

Modification of ECM proteins may also represent an important event in chronic stimulation of biochemical pathways such as protein kinase C and NF-κB (Fig. **3**). One consistent and continual structural change that accompanies diabetes *in vivo* and exposure to glucose *in vitro* is ECM protein deposition. Experimental evidence shows that the amount and the composition (type of ECM proteins) of ECM changes in diabetes. This matrix serves as a reservoir of growth factors and other signalling proteins. With continued exposure to high levels of glucose, the ECs may accumulate growth factors and other mitogens in the matrix. These accumulated factors may provide continual signaling to the ECs. In fact, ECs exposed to glucose for more than 72 hours have been shown to increase protein levels of VEGF [111]. We have also shown that the mRNA of VEGF is upregulated as early as 24 hours following exposure to high levels of glucose [89]. In addition to providing growth factors itself as a reservoir, the changing matrix may also regulate how ECs perceive and respond to the growth factors [75].

Finally, the ECM may also be modified by glycation. Glycation may decrease the turnover rate of the proteins and thus provide an efficient means of chronic signaling to the vascular ECs. Glucose reacts with proteins *via* Maillard reaction to produce advanced glycation end products (AGEs) [112] (Fig. **1**). AGE products have also been reported to cause NO scavenging [113]. Furthermore, AGE may interact with cell surface receptors (RAGEs) to increase cytokine/growth factor expression [114].These findings support the notion that ECM is equally if not more important than growth factor alteration and may provide a more sustained signaling environment.

KEY CONCEPTS AND FUTURE DIRECTIONS

The hypothesis that diabetic complications arise as a result of EC dysfunction is increasing being acclaimed. A number of biochemical variables are involved which dictate tailored therapy for the prevention as well as restoration of the EC function in diabetes. Acute effects of hyperglycemia may be solely mediated by metabolic changes (polyol pathway, hexoseamine pathway), vascular tone (vasoactive factors), and oxidative stress. Patented agents targeting some of these altered pathways have shown promise in terms of delaying the progression of the complications. However, the key elements which still require clarification are: a) the factors involved in the late stages of the disease, b) the mechanisms underlying the promotion of blood vessels in some target organs while suppressing the process in other organs, and c) the molecular signature of diabetic complication. Deciphering these key concepts may provide the best therapeutic targets. Towards this aim, studies currently being undertaken will highlight the the precise role of ECM/BM changes (tissue environment), response of accessory cells, vasoactive factors, and oxidative stress in EC biology and diabetic complications. The restoration of the EC function may well become a pre-requisite for therapy development (please see Tables **2** and **3**). A possible avenue, which still requires investigation, may be the use of combinatorial therapy. For example, use of ET receptor antagonists (bosentan), NO donors (molsidomine or other prodrugs), and oxidative stress inhibitors (may comprise of PKC, NADPH oxidase, AGE breakers, and PARP inhibitors) together may provide defense against both early and late phases of the vascular disease. We believe that targeting the EC lining in the target organs would provide the most efficient therapeutic tool for the treatment of chronic diabetic complications.

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