MINIREVIEW

Diabetic Nephropathy: Mechanisms of Renal Disease Progression

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Diabetic nephropathy is characterized by excessive amassing of extracellular matrix (ECM) with thickening of glomerular and tubular basement membranes and increased amount of mesangial matrix, which ultimately progress to glomerulosclerosis and tubulo-interstitial fibrosis. In view of this outcome, it would mean that all the kidney cellular elements, *i.e.*, glomerular endothelia, mesangial cells, podocytes, and tubular epithelia, are targets of hyperglycemic injury. Conceivably, high glucose activates various pathways *via* similar mechanisms in different cell types of the kidney except for minor exceptions that are related to the selective expression of a given molecule in a particular renal compartment. To begin with, there is an obligatory excessive channeling of glucose intermediaries into various metabolic pathways with generation of advanced glycation products (AGEs), activation of protein kinase C (PKC), increased expression of transforming growth factor-β (TGF-β), GTP-binding proteins, and generation of reactive oxygen species (ROS). The ROS seem to be the common denominator in various pathways and are central to the pathogenesis of hyperglycemic injury. In addition, there are marked alterations in intraglomerular hemodynamics, *i.e.*, hyperfiltration, and this along with metabolic derangements adversely compounds the hyperglycemia-induced injury. Here, the information compiled under various subtitles of this article is derived from an enormous amount of data summarized in several excellent literature reviews, and thus their further reading is suggested to gain in-depth knowledge of each of the subject matter. Exp Biol Med 233:4–11, 2008

Key words: hyperglycemia; advance glycation products; protein kinase C; GTP binding proteins; reactive oxygen species; hypertension

Introduction

Diabetic nephropathy is one of the major "microvascular" complications of diabetes. The renal lesions, whether related to type 1 or 2 diabetes mellitus, are similar (1). Various cells involved include glomerular podocytes, mesangial and endothelial cells, tubular epithelia, and interstitial fibroblasts and vascular endothelia. The pathophysiologic changes in diabetic nephropathy include hyperfiltration and microalbuminuria followed by worsening of renal functions associated with cellular and extracellular derangements in both the glomerular and tubulo-interstitial compartments (2). They include hyperplasia/ hypertrophy of various cell types of the glomerulus and tubules, associated with thickening of glomerular and tubular basement membranes, and expansion of tubulo-interstitial and mesangial compartments (3). Other changes include hyalinization of arterioles and at times thickening of branches of intrarenal arteries that leads to impairment in "autoregulation" of glomerular microcirculation, which apparently could amplify the renal damage.

The activity of transporters in various renal cell types has not been clearly defined (4); nevertheless, upon glucose entry the intracellular signaling events may be similar in most of them. However, one may expect differential handling of glucose intermediaries depending on a given molecule expressed in a particular cell, *e.g.*, aldose

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reductase and *myo*-inositol oxidase in the tubular cells and PKC-α and -β isoforms in the glomeruli. In general, the cellular events include increased flux of polyols and hexosamines, generation of advanced glycation end products (AGEs), increased activity of protein kinase C (PKC), TGF-β-Smad-MAPK and G-proteins, altered expression of cyclin kinases and their inhibitors, and of matrix degrading enzymes and their inhibitors with a conceivable common signaling denominator as ROS and a final outcome of increased synthesis and deposition of extracellular matrix (ECM) (5–7). The ROS, whether mitochondrial or cell membrane–derived, may also be responsible for the activation of "renin-angiotensin system" (RAS), contributing further compromise in the renal functions. As suggested by recent studies it is conceivable that there may be a cross-talk between the common metabolic denominator, *i.e.*, ROS and RAS, and both in synchrony may amplify signaling events (8, 9) (Figure 1), and this review briefly summarizes various pathogenetic mechanisms and pertinent molecules involved in the progression of diabetic nephropathy.

Channeling of Glucose in Various Metabolic Pathways. Various transmembrane proteins serving as transporters translocate glucose into the cells (4). Interestingly, albeit normal glucose ambience, there is an excessive production of ECM by GLUT-1-overexpressing mesangial cells. On the other hand, a decreased synthesis of fibronectin is seen with gene disruption of GLUT-1 *in vitro*, suggesting their role upstream of various cellular events described below (4). Following glucose entry, it first is phosphorylated and then converted to fructose 6-phosphate and glyceraldehyde 3-phosphate (G3-P) (10). By action of various transferases and phosphatases G3-P forms glycerol phosphate, a precursor of diacylglycerol (DAG) and a well-known signaling molecule (*vide infra*) (11). The fructose 6-phosphate is also converted to glucosamine-6-phosphate by glutamine:fructose-6-phosphate-aminotransferase. The latter modulates promoter activities of ECM modulating TGF-β1 and PAI-1 by phosphorylating transcription factor Sp1 (3). Under high glucose ambience, there is an increased activity of accessory polyol pathway; as a result, the glucose is reduced to sorbitol by a NADPH-dependent enzyme, aldose reductase (AR) (12). The sorbitol is oxidized to fructose by sorbitol dehydrogenase utilizing NAD^+ as a co-factor. This leads to relative depletion of NADPH and reduced glutathione (GSH), an increase in the $NADH/NAD^+$ ratio, and decreased levels of nitric oxide (NO), leading to altered cellular redox and oxidant and osmotic stress. The relevance of polyol pathway in diabetic lesions *per se* is unclear; however, reduced levels of GSH in the eye lens in mice overexpressing AR have been reported (12). Another enzyme pertinent to glucose metabolism is *myo*-inositol oxygenase (MIOX) (13). It is expressed in the tubular epithelium and is responsible for the oxidation of *myo*-inositol generated from glucose 6-phosphate after a series of steps. Phosphatidylinositol, a metabolic product of *myo*-inositol is involved in cellular signaling and osmoregulatory functions of the kidney. MIOX expression is up-regulated in experimental diabetes, while renal concentration of *myo*-inositol decreases, and its supplementation normalizes glucose-induced proliferation and collagen synthesis in tubular cells (14), thus implicating its role in diabetic nephropathy.

Relevance of AGEs in the Pathogenesis of Diabetic Nephropathy. AGEs are heterogeneous groups of macromolecules that are normally formed non-enzymatically by the interaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids, but their formation increases under high glucose ambience. Initially a labile Schiff base is formed that undergoes a series of chain reactions, *i.e.*, Amadori rearrangement, dehydration, polymerization, and ultimately AGEs' formation. The AGEs are formed both extracellularly from glucose and intracellularly from various dicarbonyls. The extracellular AGEs interact with their receptor, RAGE, and other binding proteins, *i.e.*, OST-48, 80K-H, galectin-3, and induce various intracellular events (7). The intracellular AGEs also initiate several signaling events by activating PKC, MAP kinase, and transcription factors such as NF-κB. This would increase the activity of various growth factors, such as TGF-β, and thereby alter expression of ECM proteins (15). The latter include increased synthesis of type I and IV collagens, decreased expression of proteoglycans, and anomalous ECM polymerization and expansion. Intriguingly, the AGEs themselves can covalently bind with proteins, thus compounding their deleterious effects in various tissues (15). In addition, there are perturbed cell:matrix interactions, altered adhesiveness, and capillary permeability. These ECM-vascular abnormalities are partially reversed by interrupting AGE:RAGE interactions (16). Another important AGEs-induced cellular event includes formation of reactive oxygen species (ROS) that as well modulates the activity of various kinases and transcription factors that ultimately contribute to ECM pathology (7, 17).

Activation of PKC and Ensuing Various Cellular Signaling Events. Among various signaling kinases, PKC seems to be a centerpiece in the pathogenesis of diabetic nephropathy (17). Under high glucose ambience it is activated by DAG formed during glycolytic intermediary steps and by ROS generated following AGE:RAGE interactions (7, 18). Such interactions at the cell membrane activate PKC by increasing the activity of phospholipase C with an increase in intracellular Ca^{2+} and DAG. This cyclic generation of DAG would suggest an intimate "level and activity" relationship between DAG and PKC, and such a parallelism in their expression has been observed in various tissues in diabetes (18). The PKC activation also leads to endothelial dysfunction with decreased nitric oxide production, increased expression of endothelin-1, and vascular endothelial growth factor. This would alter blood flow and capillary permeability that are compounded by increased TGF-β1–induced synthesis of integral ECM proteins of the vasculature (18). At the same time, increased expression of NF-κB and PAI-1 would induce a local tissue inflammatory

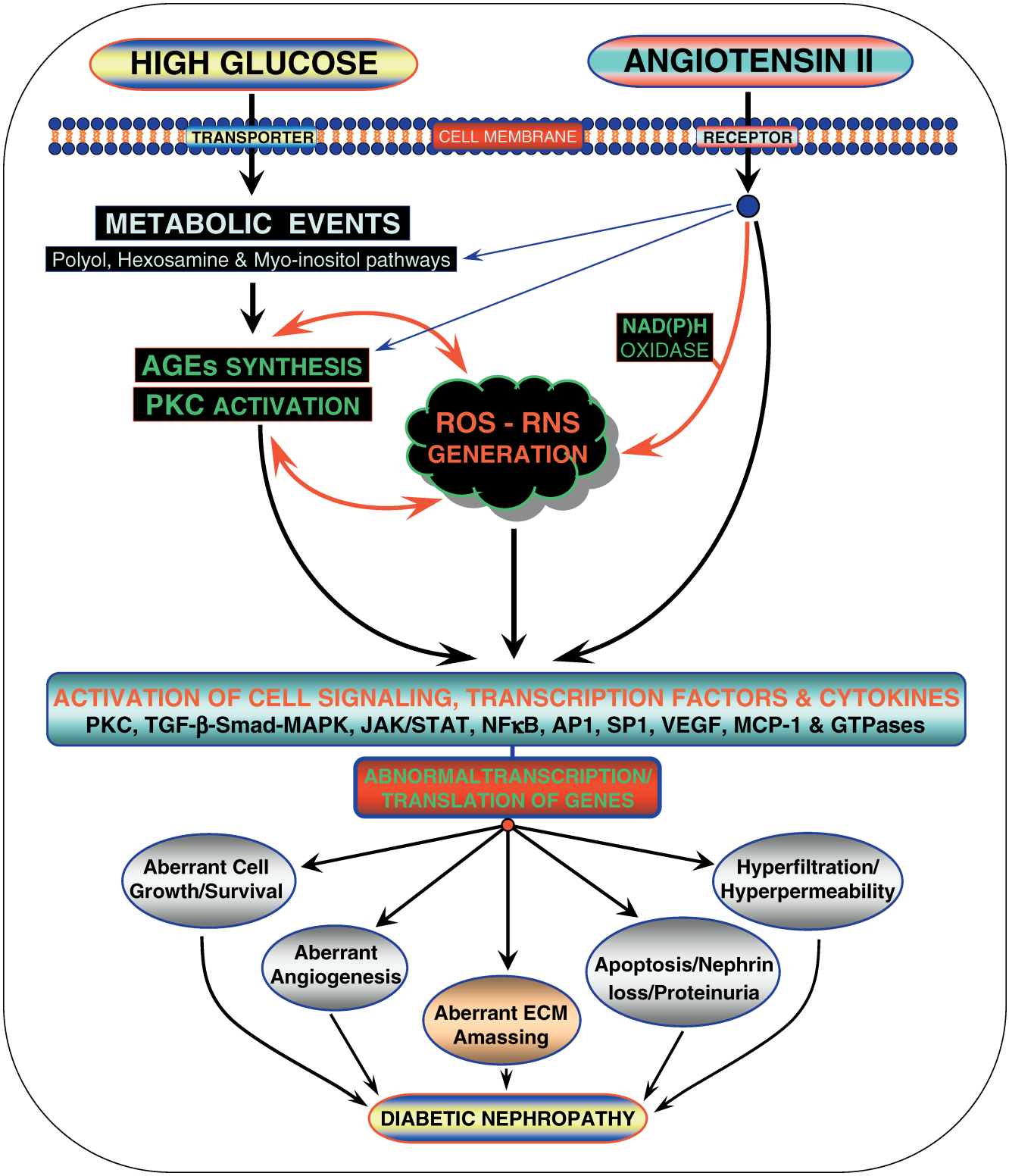

Figure 1. Various mechanisms relevant to hyperglycemia-induced and angiotensin II–induced activation of different signaling pathways with altered expression of various genes and cellular dysfunctions, leading to diabetic nephropathy and chronic renal failure.

response and thrombotic microangiopathy, thus accentuating the vascular injury, which is further augmented by additional ROS generated by plasmalemmal NADPH oxidoreductase (7). The fact that PKC activation occurs by multiple routes and that the administration of PKC inhibitor, ruboxistaurin mesylate, reduces renal abnormalities in *db*/*db* mice suggests its central signaling role in hyperglycemia-induced vascular injury (7, 18).

Cellular Pathways Induced by TGF-β. The AGEs, ROS, DAG, PKC, and hexosamines are the potential candidates that activate TGF-β signaling (19), while others compounding the hyperglycemic injury include vasoactive substances, *i.e*, angiotensin II, endothelin, and thromboxane, and cyclical stretch and relaxation of mesangial cells mimicking intraglomerular hypertension (20). The final outcome would be aberrant accumulation of ECM proteins, which is further facilitated by an inhibition of matrix proteases (MMPs) and activation of the corresponding inhibitors (TIMPs). At present, TGF-β is recognized as the major cytokine responsible for ECM pathobiology seen in diabetic nephropathy (21). Initially, TGF-β binds to a type II receptor, which trans-phosphorylates type I serine/threonine kinase receptor. The latter interacts with Smad2 and 3, which then forms a complex with Co-Smad4 (22). Following nuclear translocation of this complex, it binds with promoters of TGF-β target genes, *e.g*., collagen α1(I), PAI-1, Jun B, c-Jun, and fibronectin and regulates their transcription. These events are negatively regulated by Smad7. Besides Smads, the mitogen activated protein kinases (MAPKs) involved in hyperglycemia-induced TGF-β signaling include extracellular signal regulated kinases 1 and 2 (ERK1 and 2, p44/p42 MAPKs), c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), and p38 MAP kinase (22). The MAPKs also modulate transcriptional regulation of ECM genes conceivably *via* activated protein-1 (AP-1), a heterodimer of c-Fos and c-Jun. Since AP-1, regulated by ERK and JNK, can bind to Smad3 while Smad3:Smad4 complex to AP-1 consensus sequences in various promoters of TGF-β target genes, this would suggest a cross-talk between Smad and MAPK pathways activated by high glucose ambience (22). Another cytokine relevant to TGF-β signaling is connective tissue growth factor (CTGF) that is induced by TGF-β *via* consensus Smad and transcription enhancer factor (TEF) elements localized within the CTGF promoter (23). The above alluded signaling has been worked out in mesangial cell culture systems, and its *in vivo* relevance is also well-described in mice models of diabetes, where heightened TGF-β bioactivity has been observed. Moreover, administration of neutralizing anti–TGF-β antibodies prevents renal hypertrophy, mesangial matrix expansion, increased collagen and fibronectin mRNA expression, and deterioration of renal functions in *db*/*db* or STZ-induced diabetes in mice (21). Likewise, its upregulated tissue expression and increased urinary excretion of TGF-β in patients with diabetic nephropathy has been observed. Interestingly, angiotensin-converting enzyme inhibitors that ameliorate renal damage concomitantly also lower the TGF-β production, suggesting a relationship of hypertension and ECM pathobiology in diabetes mellitus (20).

Mechanisms Related to Generation of ROS. The ROS seem to be the common denominators and amplifiers of the above-described signaling cellular pathways activated by hyperglycemia (5, 7, 24). They are continuously produced and degraded normally to maintain homeostasis, but when generated at high concentrations, such as in hyperglycemia, they are capable of inducing injury in various organs (25). The ROS that can induce renal injury include superoxide anion ($O_2^{\cdot-}$), H_2O_2, hydroxyl radical, and peroxynitrite (26). Their redox balance is maintained by various enzymes, including cytoplasmic Cu/Zn superoxide dismutase (Cu/ZnSOD) and mitochondrial manganese SOD (MnSOD), the most important being heme oxygenase-1(HO-1) that undergoes remarkable induction by hyperglycemia (27). Most of the ROS are generated during mitochondrial oxidative phosphorylation and small amounts *via* NADPH-oxidase system (28–30).

During oxidative phosphorylation the electron donors generate a high membrane potential by pumping protons across the mitochondrial inner membrane (5, 31). As a consequence, the electron transport is inhibited and the half life of free-radical intermediates of ubiquinone increases, which reduces O_2 to $O_2^{\cdot-}$ with ensuing oxidant stress. Rotenone, an inhibitor of electron transport, blocks the DCF-sensitive ROS generation, and the fact that a similar effect is observed by overexpression of mitochondrial MnSOD or uncoupling protein-1 (UCP-1) supports this notion (5, 32). Conceivably, the MnSOD or UCP-1 reverses the oxidant stress generated by the high glucose-induced cellular events, including the polyol and hexosamine fluxes, AGE formation, and PKC activation. Whereas, $O_2^{\cdot-}$ generated initially (*vide supra*) inhibits glyceraldehyde-3-phosphate dehydrogenase (GAPDH) that in turn amplifies these cellular events with repetitious generation of ROS (24). Similarly, since gene disruption of GADPH also amplifies the above cellular event it would support the role of mitochondrial ROS in hyperglycemia-induced injury and ECM accumulation. Along these lines, it is interesting to note that the overexpression of extra-mitochondrial cytoplasmic Cu/ZnSOD reduces the glomerular pathophysiologic changes in *db*/*db* mice and STZ-induced diabetes (33, 34).

The ROS are generated *via* NADPH-oxidase system by the interaction of membrane-bound flavocytochrome b_{558} (heterodimer of $gp91^{phox}$ and $p22^{phox}$) with various cytosolic proteins ($p47^{phox}$, $p67^{phos}$, $p40^{phox}$, and a GTP-binding protein, p21rac) (29, 35); as a result $O_2^{\cdot-}$ is generated, which gets dismutated to H_2O_2. The relevance of this system in renal pathobiology lies in the fact that Nox 4, a homologue of neutrophil $gp91^{phox}$, is expressed in the kidney (29). Besides AGEs, PKC, DAG, IP3 (inositol 1, 4, 5-trophosphate), and TGF-β the metabolites of cyclo-oxygenase (COX) pathway and can also activate NADPH oxidase under high glucose ambience (25, 32). Moreover, decreased fibronectin expression by the inhibitors of oxidase, apocynin, and diphenylene iodonium (DPI) suggests a potential role of NADPH oxidase in hyperglycemic injury and relevance in redox-sensitive processes, *i.e*., cell growth, apoptosis, migration, and extracellular matrix (ECM) modeling that are modulated by various signaling pathways and transcription and growth factors such as TGF-β. The latter adversely affects the

biology of tubular cells with induction of epithelial–mesenchymal transition, and loss of epithelial cell adhesion, α-smooth muscle actin expression, cytoskeletal organization, and cell migration through the basement membrane and ensuing of tubulo-interstitial fibrosis (36). Other systems in which ROS are generated include nitroso-redox balance, where reactive nitrogen species (RNS), *e.g.*, derivatives of NO, are produced (37). Briefly, NO synthesis is modulated by a cofactor of nitric oxide synthase (NOS), known as tetrahydrobiopterin (BH4) (38). BH4 levels are reduced in high glucose ambience with reduced synthesis of NO by the endothelium, leading to an altered ratio of BH4 and its oxidized form, BH2, with increased generation of superoxide (39). Further support for the role of RNS in pathogenesis of hyperglycemic injury is derived from the studies elucidating an amelioration of endothelial/smooth muscle dysfunction in diabetic rat aortic rings exposed to BH4 (39).

Amplification of Hyperglycemic Injury by Glomerular Capillary Hypertension. Like the ROS that amplify the hyperglycemic injury, hypertension, or, more importantly, the increased glomerular capillary pressure also significantly contributes to the acceleration of diabetes-related complications, perhaps *via* a cross-talk between metabolic and hemodynamic factors being operative under high glucose ambience—a concept that has emerged in recent years and deserves much attention (40). The evidence for intraglomerular hypertension was derived from micropuncture studies in experimental diabetes, and interestingly the systemic blood pressure was found to be normal in these animals (41). This implied that hyperglycemia impairs "autoregulation" of local glomerular microcirculation with dilatation of arterioles, more so of the afferent arteriole, thus affecting the transcapillary hydraulic pressure difference and plasma flow (41). Conceivably, hyperglycemia sensitizes the target organs to blood pressure–induced damage, most likely by activation of renin-angiotensin system (RAS) with local production of angiotensin II (Ang II) in the kidney (9). The support for this notion comes from studies in which lowering of the blood pressure had comparable or even more beneficial effects, *e.g.*, on microalbuminuria, than controlling hyperglycemia (42). The cells that may be involved in the activation of local RAS include proximal tubular epithelia, glomerular mesangial cells, and podocytes; the latter apparently also produce Ang II and express AT1 receptors (43, 44). Hence, it seems that due to some error of evolution the stage is set in which the kidney would be a bidirectional target of hyperglycemia-induced as well as pressure-induced injury in a diabetic state, although one may be a consequence of the other; meaning thereby that inhibition of angiotensin converting enzyme (ACE) would be an integral part of the therapy for amelioration of diabetic nephropathy. In this regard, it is worth mentioning that ACE gene insertion/deletion (I/D) polymorphism studies indicate that individuals with ACE D/D genotype respond poorly to ACE inhibition, suggesting that in this quandary of metabolically induced altered hemodynamics the genetics also have a considerable influence in the ultimate outcome of this disease (45). Along these lines, ACE2 knockout mice have been reported to develop glomerulosclerosis that is reminiscent of diabetic nephropathy (46). Similarly, deficiency of ACE2, a negative regulator of ACE, is associated with a local increase of tubular Ang II and tubulointerstitial fibrosis in long-term experimental diabetes (47).

With respect to the pathogenesis of diabetic nephropathy, the relevant question would be as to what are the mechanism(s) by which glucose sensitizes the kidney to pressure-induced damage *via* the generation of Ang II, which is central to early hyperplasia and late hypertrophy of the renal cells with upregulation of various cytokines, *e.g.*, TGF-β, CTGF, IL-6, monocyte chemotactic protein-1 (MCP-1), and vascular endothelial growth factor (VEGF), that is followed by accumulation of the ECMs (8, 9, 41). To begin with high glucose has been shown to increase the expression of renin and angiotensinogen (AGT) in mesangial and tubular cells, which could increase intrarenal concentration of Ang II and then *via* various autocrine and paracrine pathways lead to generation of various cytokines and ECM accumulation (48). Secondly, in review of the metabolic events discussed earlier (*vide supra*) it is conceivable that glucose-mediated generation of ROS *via* AGE:RAGE interaction could also upregulate the expression of AGT and Ang II in tubular cells (7, 49). Similarly, infusion of AGEs *in vivo* results in a remarkable increase in the expression of various components of the RAS (49). Interestingly, the infusion of Ang II increases both serum and renal accumulation of AGEs, thus highlighting autocrinicity and complexity of the events in a *diabetic milieu* (49). In terms of autocrinicity the subject matter of discussion would be to address the mechanisms by which altered intraglomerular hemodynamics amplify hyperglycemia-induced renal injury. An expected effect of increased capillary pressure would be a stretch-stress on the glomerular cells that parallels to changes in glomerular volume and activation of various signaling pathways.

The mechanical stretch has a profound effect on the pathobiology of mesangial cells. First of all, *in vitro* studies indicate that such a stress induces expression of GLUT-1, which certainly would lead to an increased cellular concentration of glucose and activation of various pathways (50). Secondly, repetitive cyclic stretch/relaxation of mesangial cells has been shown to enhance their proliferation and increase synthesis of ECM while decreasing expression of ECM-degrading enzymes (51). Increased ECM synthesis may in part be due to the direct mechanical stretch-induced expression of TGF-β and its receptors as well. The TGF-β activation in this scenario could also be due to stretch-induced activation of PKC and p38 MAPK with consequential increased synthesis of fibronectin (51). Ang II mediated stretch-stress in mesangial cells also induces the expression of an inflammatory cytokine, MCP-1 (52). As a consequence an upregulation of intercellular adhesion molecule-1 (ICAM-1) and generation of ROS

would be anticipated with amplification of the inflammatory response and renal injury. In support of such anticipation are renal biopsy studies, which at times show an influx of monocytes in patients with diabetic nephropathy (53). The stretch-stress also modulates the biology of glomerular podocytes, conceivably *via* elaboration of VEGF (54). These podocytes undergo reversible reorganization of the cytoskeletal elements and reduced α3β1 integrin; as a result the cells detach from the underlying GBM and undergo apoptosis (54). Another interesting aspect of the podocyte biology is that the expression of nephrin, a slit membrane protein, is reduced, conceivably *via* VEGF signaling, with expected increased urinary excretion of proteins in diabetic state (54). Intriguingly enough, decreased expression of nephrin and relative loss of podocytes is also observed in offsprings in states of maternal hyperglycemia, and it has been postulated that this underdosing of nephrons or podocytes during fetal development may be a susceptibility factor to develop hypertension in later adult life (55). Overall, it appears that the metabolic and hemodynamic events are interlinked in states of hyperglycemia with a certain degree of influence of genetic background.

Pathogenetic Mechanisms Related to GTP-Binding Proteins and Cell Cycle Proteins. The role of GTP binding protein is not well-appreciated, but certainly deserves a brief discussion. Major GTP proteins activated in hyperglycemic state belong to the Ras and Rho family of small GTPases (56). They exert pleiotropic effects, *i.e.*, regulate growth, morphogenesis, cell motility, axonal guidance, cytokinesis, and intracellular trafficking by cycling between inactive (GDP-bound) and active (GTP-bound) state. The Ras and Rho are of special significance since they transduce signals from extracellular stimuli to intracellular signal transduction pathways relevant to the pathobiology of diabetes. For instance, in Ras/Raf/MEK signaling, the Ras acts as an intermediary between the phosphorylated growth factor receptor and MEK (MAP kinase/ERK) activation. Whereas, Rho and Rho-related Rac can directly activate SAPK/JNK and p38 MAPK pathways, respectively (57). The activation of Ras-related Rap1 occurs upon binding with Raf1, which itself is activated by a phosphorylated form of growth factor receptor. The Rap1 can also be induced by intracellular second messengers, *e.g.*, DAG, which can directly or *via* PKC also activate the Rap/Raf/MAPK pathway under high glucose ambience (58, 59). The activated Rho-induced ECM fibronectin synthesis in renal cells most likely is modulated by the TGF-β–mediated upregulation of CTGF and also by other known profibrogenic molecules, including angiotensin II, PDGF, and endothelin-1 (60). The Rho GTPases–induced SAPK/JNK kinases also activate a number of transcription factors, including cAMP-responsive element binding protein (CREB). Since cis-acting cAMP responsive elements TGACGTCA are present in the fibronectin promoter, the CREB may be the key transcription factor that regulates ECM protein expression in diabetic *milieu*. Finally, post-translational modifications, such as farnesylation or geranylgeranylation of GTPases, may be responsible for their activation with increased synthesis of ECM proteins (61).

Other cellular processes modulated by GTPases include cellular proliferation by activating SAPK/JNK and p38 MAPK pathways like TGF-β under high glucose ambience (56, 62). The proliferation of mesangial cells, as seen in initial stages of diabetic nephropathy, is modulated by cell cycle proteins, cyclin and cyclin-dependent kinases (CDK), and their inhibitors, CDIs (61, 63, 64). The latter include 2 major groups, *i.e.*, Cip/Kip ($p21^{Cip1}$and $p27^{Kip1}$) and 1NK4/ARF ($p16^{1NK4}$ and $p14^{ARF}$). The expression of $p27^{Kip1}$ of Cip/Kip is increased in the kidneys of *db*/*db* and streptozotocin-induced diabetes in mice and mesangial cells exposed to high glucose ambience. Conceivably, this increased expression is related to $p27^{Kip1}$ phosphorylation since MAP kinase and PKC are activated in diabetic *milieu*. Interestingly, gene disruption of $p27^{Kip1}$ results in decreased protein synthesis, suggesting conversion from hypertrophic to a hyperplastic state of cells exiting the G1 phase of the cell cycle. Similarly, induction of diabetes in $p27^{Kip1}$-/- or $p21^{Cip1}$ -/- mice does not exhibit any hypertrophic response, but hyperplasia of renal tubular cells. Typically, the $p27^{Kip1}$-/- mice do not develop diabetic nephropathy as no renal or glomerular hypertrophy develops (65). With respect to $p21^{Cip1}$, its decreased expression is associated with reduced CDK-2 and -4 activities and increased expression of Ras and Rho in proliferating cells exposure to high glucose, thus establishing a link between activated GTPases and cell cycle proteins (61).

In summary, mechanisms that are relevant to the pathogenesis of diabetic nephropathy are discussed. They involve various signaling pathways modulated by large number molecules regulating the bioactivity of one another and at times amplifying via autocrine loops with a final outcome of amassing of ECM in various compartments of the kidney as characteristically seen in diabetic nephropathy. A more recent interesting concept that seems to enjoy a universal consensus has emerged which implicates that both the metabolic and hemodynamic events are interwoven together in the pathogenesis of diabetic nephropathy. Still, further advancements are necessary to delineate other cellular pathways modulated by known or unknown genes to gain new insights into its pathogenesis. In this regard, mitochondrial genome needs to be searched for mutations in the genes that could be relevant to ROS and oxidative phosphorylation and perturb the biology of target cells. Similarly, the understudied subject of the role of GTP-binding proteins in the hyperglycemia-induced cellular changes needs further attention as well.

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