

# Role of Protein Kinase C-MAPK, Oxidative Stress and Inflammation Pathways in Diabetic Nephropathy

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## Abstract

Diabetic nephropathy is the most common cause of end-stage renal disease. It is assumed that hyperglycemia is one of the major systemic risk factors for diabetic complications. Numerous hypotheses exist to enlighten the adverse effect of hyperglycemia. One of these hypotheses is the activation of the calcium- and phospholipid-dependent protein kinase C signaling pathway by hyperglycemia which subsequently mediates cellular response and affects gene expression and protein function to cause cellular dysfunction and damage. It is well known that the intracellular protein kinase C activation is achieved by the elevated diacylglycerol levels in vascular tissues as well as in nonvascular tissues. Besides diacylglycerol, oxidative stress has also been reported to induce prolonged activation of protein kinase C within cells through the reactive oxygen species. Activation of protein kinase C and oxidative stress have been associated with vascular alterations such as increases in permeability of endothelial cells, extracellular matrix synthesis, cell growth and apoptosis, angiogenesis, and cytokines activation and inhibition. These derangements in vascular cell homeostasis caused by the activation protein kinase C as well as oxidative stress are connected to the occurrence of pathologies affecting large vessel and small vessel complications. Accumulating evidences have also shown that the inflammation process is an essential pathogenetic mechanism in diabetic nephropathy. Therefore, modulation of this process is an important target for both metabolic and hemodynamic derangements in diabetic nephropathy. In this review, we will discuss the role of protein kinase C, oxidative stress and inflammation process and the signaling pathway in the pathogenesis of diabetic nephropathy.

## Introduction

Diabetes mellitus (DM) is a complex syndrome characterized by absolute or relative insulin deficiency leading to hyperglycemia and an altered metabolism of glucose, fat, and protein. The major cause of metabolic dysfunction in DM is its complications, which are the result of interaction among systemic metabolic abnormalities such as hyperglycemia, dyslipidemia, and local tissue responses to toxic metabolites. Complications involve large vessel obstructions, such as coronary artery diseases and microvascular pathologies, such as retinopathy, neuropathy, and nephropathy. Accordingly, patients with diabetes have a much higher risk of myocardial infarction, stroke and limb amputation. Large prospective clinical studies show a strong relationship between glycaemia and diabetic microvascular complications in both type 1 and type 2 [1,2].

Diabetic nephropathy (DN) is a leading cause of chronic kidney disease that progresses to end-stage renal disease (ESRD) and affects 30% of patients with type 1 DM and up to 25% of all patients with type 2 DM. DN is an extremely frequent complication of DM that profoundly contributes to patient morbidity and mortality [3-5]. The pathophysiologic changes in DN include the occurrence of persistent microalbuminuria and hyperfiltration followed by a hyperplasia/hypertrophy of various cell types of the glomerulus and tubules and associated with the thickening of glomerular and tubular basement membranes as well as accumulation of extracellular matrix (ECM) components. Other changes include hyalinization of arterioles and thickening of branches of intrarenal arteries that leads to the impairment in autoregulation of glomerular microcirculation, which could augment the renal damage [6,7]. Advanced DN is frequently

characterized by diffuse glomerulosclerosis and may sometimes exhibit a distinctive morphological appearance, that is, the nodular form of glomerulosclerosis, as first described by Kimmelstiel and Wilson in 1936 [8]. Several mechanisms contribute to the development of DN, including an interaction between metabolic abnormalities, hemodynamic changes, genetic predisposition, inflammatory milieu and oxidative stress constituting a continuous perpetuation of injury factors for the initiation and progression of DN [9].

Multiple biochemical pathways have been proposed to connect the adverse effects of hyperglycemia with vascular complications. Although a single theory has not been established to explain all these changes, a few has emerged that can include most of the data that have accumulated in this area. Hyperglycemia can lead to the activation of several cellular pathways, including increased activation of the polyol pathway flux (in which glucose is reduced to sorbitol, lowering

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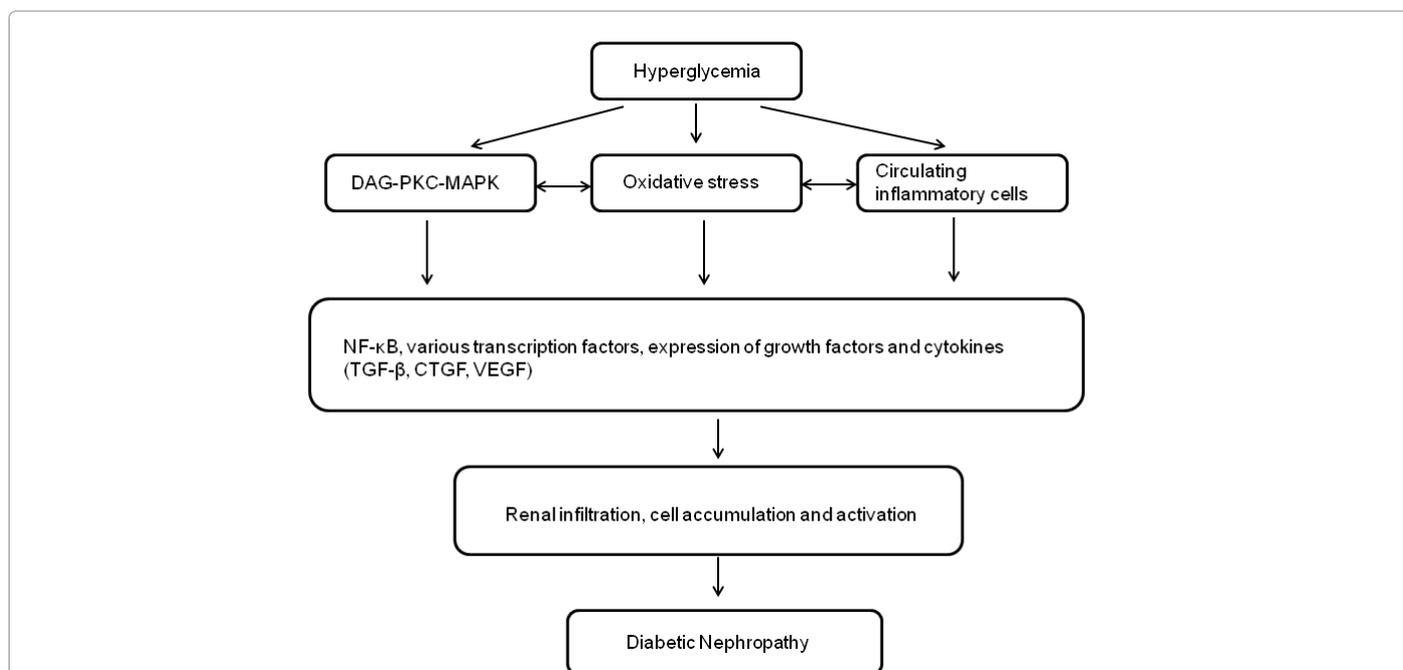
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levels of both reduced nicotinamide adenine dinucleotide phosphate (NADPH) and reduced glutathione) [10], increased advanced glycation end-product (AGE) formation [11], increased shunting of excess glucose through the hexosamine pathway (mediating increased transcription of genes for inflammatory cytokines) [12], and activation of the diacylglycerol (DAG)-protein kinase (PK) C-mitogen-activated protein kinase (MAPK) pathways (effects ranging from vascular occlusion to expression of proinflammatory genes) [13-15]. All of these biochemical changes are activated by a common mechanism, that is, overproduction of superoxide radicals and associated oxidant stress [16], which can ultimately lead to increased formation of ECM proteins in the kidney, contributing to renal dysfunction [17]. The DAG-PKC pathway has been shown to be important in vascular cells to regulate permeability, contractility, ECM, cell growth, angiogenesis, cytokine actions and leukocyte adhesion [18,19]. Accumulating evidences have also demonstrated that high glucose can activate the proinflammatory transcription factor NF- $\kappa$ B, resulting in increased inflammatory gene expression [20-23]. As suggested by previous studies, it is conceivable that there may be a crosstalk between the PKC-MAPK pathways, oxidative stress and inflammation process, and all are in synchrony may amplify signaling events to cause DN [24-26] (Figure 1). In this review, we discuss the role of PKC actions, oxidative stress and inflammatory process as well as related signaling cascade in the development of DN.

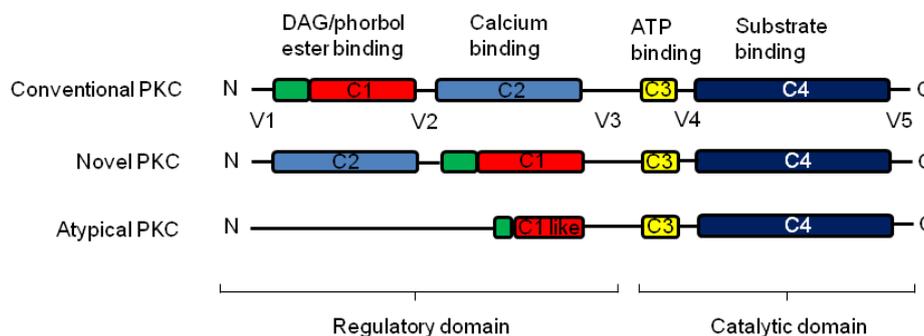
### PKC at a glance

PKC is a serine/threonine-related protein kinase that plays a key role in many cellular functions and affects many signal transduction pathways [27]. Coordinated regulation of this enzyme activation is crucial for normal cell functions; in contrast, unusually persistent activation of PKC may lead to uncontrollable growth. Biochemical

and molecular cloning analysis have revealed that the enzyme comprises a large family with multiple isoforms exhibiting individual characteristics and distinct patterns of tissue distribution [28]. These kinases contain a highly conserved C-terminal catalytic domain (consist of motifs required for ATP/substrate-binding and catalysis) and a regulatory domain that maintains the enzyme in an inactive conformation. The regulatory domains reside in the NH<sub>2</sub> terminus of the protein and contain an autoinhibitory pseudosubstrate domain and two discrete membrane targeting modules, which is C1 and C2 [29]. To date, 12 PKC isozymes have been identified and classified into three groups based on differences in their NH<sub>2</sub>-terminal regulatory domain structure [19]. The first discovered is the conventional PKCs ( $\alpha$ ,  $\beta_1$ ,  $\beta_2$ ,  $\gamma$ ), which are activated by phosphatidylserine, calcium, and DAG or phorbol esters such as phorbol 12-myristate 13-acetate (PMA). The next well characterized are the novel PKCs ( $\delta$ ,  $\epsilon$ ,  $\theta$ ,  $\eta$ , and  $\mu$ ), which are activated by phosphatidylserine, DAG or PMA, but not by calcium. The last isozymes are the atypical PKCs ( $\zeta$ ,  $\mu$  and  $\lambda$ ), which are not activated by calcium, DAG, or PMA (Figure 2). Traditionally, the activation of calcium-dependent PKC isoforms involves the hydrolysis of phosphatidylinositides (PI) and generation of inositol-(1,4,5)-triphosphate (IP<sub>3</sub>) which lead the mobilization of intracellular calcium, a soluble ligand that binds to the C2 domain and increases its affinity for membranes. Once anchored to membranes, the calcium-dependent PKC isoforms diffuses within the lipid bilayer and participates in a secondary C1 domain interaction with DAG which in turn leads to a conformational change of calcium-dependent PKC isoforms that expels the autoinhibitory pseudosubstrate domain and facilitates PKC activation [29]. PKC translocation to the plasma membrane generally has been considered the hallmark of activation and has been used as a surrogate measure of PKC isoform activation in cells.



**Figure 1:** Hyperglycemia-induced activation of molecular pathways associated with diabetic complications. Diabetes and associated hyperglycemia can lead to increased activation of diacylglycerol (DAG)-protein kinase C (PKC)-mitogen activated protein kinase (MAPK), oxidative stress and circulating inflammatory cells. All of these events can lead to production and increased action of various growth factors and cytokines such as transforming growth factor (TGF)- $\beta$ , connective tissue growth factor (CTGF), and vascular endothelial growth factor (VEGF) as well as activation of transcription factors such as nuclear factor (NF)- $\kappa$ B, and therefore will lead to infiltration, accumulation, and activation of extracellular matrix proteins in renal tissues, all of which over time can induce the development of diabetic nephropathy.



**Figure 2:** Domain structures of classical, novel, and atypical protein kinase C (PKC). The figure shows a comparison of the protein architecture of the various subgroups of the PKC subfamily. All PKC regulatory domains have a pseudosubstrate domain (shown in green). Conventional and novel PKC contain C1 domain which mediate binding to diacylglycerol (DAG) and phorbol 12-myristate 13-acetate (PMA) and C2 domain which has a function as calcium-dependent phospholipid binding in the case of conventional PKC and a serine/threonine-kinase domain. Novel PKC C2 domains do not bind calcium. The atypical PKC, which lack the C2 region, are not activated by calcium, DAG, or PMA, but their activation depends on phosphatidylserine and *cis*-unsaturated fatty acids.

### The activation of DAG-PKC-MAPK pathway in diabetes

Increases in total DAG contents have been demonstrated in a variety of tissues associated with diabetic vascular complications, such as retina [30], aorta and heart [31], and renal glomeruli [32]. Following glucose entry into vascular and renal cells through GLUT1, it is phosphorylated and then converted to fructose 6-phosphate and glyceraldehyde 3-phosphate. By the action of various transferases and phosphatases, glyceraldehyde 3-phosphate forms glycerol phosphate, a precursor of DAG, which in turn directly or indirectly activates PKC isoforms [33,34]. The source of DAG that activates PKC can be derived from the hydrolysis of PI or from the metabolism of phosphatidylcholine (PC) by phospholipase C (PLC) or phospholipase D (PLD) [27]. Furthermore, the activation of PKC will regulate various vascular functions by modulating enzymatic activities such as cytosolic phospholipase A<sub>2</sub> and Na<sup>+</sup>-K<sup>+</sup>-ATPase, as well as gene expression of ECM components and contractile proteins [35-37]. Upon activation, PKC can transmit signals to the nucleus via different signal transduction pathways and activate MAPK through activating MAPK kinase (MAPKK).

Activation of the PKC-MAPK pathway induces enhanced ECM protein and TGF- $\beta$  expression in glomerular mesangial cells, suggesting that this pathway might be responsible for PKC-related abnormalities in diabetic glomeruli, leading to the development of DN [38-40]. From various PKC isoforms in vascular cells, PKC- $\alpha$ , - $\beta_1$ , - $\beta_2$ , - $\delta$  and - $\zeta$  appear to be preferentially activated by high glucose concentrations in various cell culture models and in the glomerular cells of diabetic rats [41-43]. Menne et al. [44] have demonstrated that diabetic PKC- $\alpha^{-/-}$  mice were protected from albuminuria by perpetuating the loss of the negatively charged heparin sulfate and upregulation of glomerular vascular endothelial growth factor (VEGF) expression [44]. Moreover, previous animal studies have shown that deletion of the PKC- $\beta$  gene or treatment with a selective PKC- $\beta$  inhibitor (LY333531 or ruboxistaurin) was associated with normalization of hemodynamic changes, ECM production, expression of connective tissue growth factor (CTGF), production of TGF- $\beta$ , and histological features of glomerular damage in animal models of diabetes [41, 45-47]. High ambient glucose-heightened extracellular signal-regulated kinase (ERK) and PKC- $\delta$  activity was shown to enhance cellular responsiveness to TGF- $\beta$  and exacerbated the production of ECM proteins by mesangial cells [48]. In

addition, high glucose-induced PKC- $\zeta$  activity was shown to mediate F-actin disassembly and alter mesangial cell contractile responses to endothelin-1 [49]. Recently, we have also shown the critical role of PKC- $\alpha$  and - $\beta_1$  isozymes-MAPK pathways in the development of DN. We demonstrated that high glucose-induced the activity of PKC- $\alpha$  and - $\beta_1$  in kidney tissues of diabetic rats and as a result, increased the protein levels of ERK1/2, VEGF, and TGF- $\beta_1$ , which were ameliorated by curcumin, a powerful antioxidant [50]. It has become evident that the aberrant activation of the PKC isozymes and other signaling mediators by glucose can alter the response of the mesangial cells to external stimuli. Such potential aberrations in cell signaling by glucose stress may have an important role in the progression of DN [48,49].

MAPK cascades comprise one of the major signaling systems by which cells transduce and integrate diverse intracellular signals. The MAPKs, including ERK 1 and 2 (p44/p42 MAPKs), c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), and p38 MAPK, are all involved in hyperglycemia-induced ECM accumulation in DN. ERKs are activated primarily in response to proliferated stimuli, whereas the other MAPKs are activated primarily in response to inflammatory and stressful stimuli, including oxidant and osmotic stress. Previous study has been demonstrated that in diabetic condition, nonenzymatic glycosylation of protein, DAG-PKC pathway, and oxidative stress could activate p38 MAPK, resulting in the phosphorylation of transcriptional factor and alteration of expression of genes, which participated in the development of DN [51]. In vitro studies have also shown that hyperglycemia could activate the p38 MAPK signaling pathway in renal cells and induced the phosphorylation of p38 MAPK in mesangial cells which promote the mesangial cells to produced fibronectin [52-55]. ERK, which is one of the MAPKs, is also an important kinase in the intracellular signal transduction system leading to cell proliferation and ECM synthesis [56]. Isono et al. [57] confirmed that ERK was activated in mesangial cells cultured in high glucose and in the glomeruli of diabetic rats [57]. Previous study has shown that high glucose-induced upregulation of ECM protein, fibronectin, occurs via activation of MAPK/ERK pathway, and this upregulation was prevented by treatment with PKC blocker, chelerythrine, which suggests that PKC is an upstream mediator of MAPK [58]. Recently, we have also demonstrated that the protein expression levels of ERK1/2, p38MAPK, and JNK were upregulated in kidney tissues of STZ-induced diabetic mice, which were attenuated by Ang-II type 1 receptor blocker [59].

## Oxidative Stress in Diabetic Nephropathy

Increased oxidative stress has been shown in patients with diabetes and has been implicated in the development and progression of diabetic microvascular complication, including DN [60,61]. Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA.

## Crosstalk between PKC and ROS

There are a number of macromolecules that have been implicated for increased generation of ROS, such as, NAD(P)H oxidase, AGE, and uncoupled nitric oxide synthase (NOS). Numerous reports have shown that NAD(P)H oxidase, which is primarily found in phagocytic cells, is the main source of ROS in nonphagocytic cells such as endothelial cells [62], adventitial cells [63], mesangial cells [64], podocytes [65], and fibroblasts [64]. NAD(P)H oxidase consists of several membrane-bound subunits, namely, gp91phox, Nox, and p22phox and cytosolic subunits, namely, p47phox and p67phox. Upon activation, some subunits of NAD(P)H oxidase are phosphorylated and translocated to the membrane by several kinases, including PKC, and form the catalytically active oxidase [66]. The significance of NAD(P)H oxidase in the pathogenesis of DN is underscored by the finding that apocynin, a pharmacological inhibitor of NAD(P)H oxidase suppresses proteinuria and mesangial matrix expansion in STZ-induced diabetic rats and in db/db mice [67,68]. Lee et al. [69] have demonstrated that NAD(P)H oxidase inhibitors, apocynin and diphenylene iodonium (DPI), and an inhibitor of mitochondrial electron transfer chain complex I, rotenone, effectively block high glucose-induced ROS generation in mesangial cells and high glucose-induced ECM protein secretion by tubular epithelial cells, which suggest that both NAD(P)H oxidase system and mitochondrial metabolism are involved in high glucose-induced diabetic vascular complications [69].

Numerous reports have suggested that PKC, such as classical PKC isozymes ( $\alpha$  and  $\beta$ ) and atypical PKC- $\zeta$ , play an important role in the activation of NAD(P)H oxidase [70-74]. Kitada et al. [73] reported that overexpression and translocation to the membrane of NAD(P)H oxidase subunits, p47phox and p67phox, were correlated with increased NAD(P)H oxidase activity, which resulted in oxidative stress in diabetic glomeruli. They also demonstrated that membranous translocation of p47phox and p67phox were dependent on the PKC- $\beta$  activation and this activation was suppressed by ruboxistaurin, a specific PKC- $\beta$  inhibitor [73].

Previous study has also shown that high glucose-induced ROS generation in mesangial cells is effectively blocked by calphostin C, a PKC inhibitor, which suggest that high glucose-induced ROS generation in mesangial cells is PKC dependent [70]. Moreover, it has reported that high glucose-induced PKC activation in mesangial cells is effectively blocked by antioxidants such as vitamin E, N-acetyl cysteine, and taurine, suggesting that there is a crosstalk between high-glucose-induced ROS and PKC activation [75].

## Role of PKC-ROS in proteinuria

DN is characterized with abnormalities in the glomerular endothelium and mesangium as well as in podocytes or glomerular visceral epithelial cells. Podocytes cover the outer aspect of the glomerular basement membrane via foot processes, and modified tight junctions between adjacent cells forms the slit diaphragm. This unique structure is specially designed to allow filtration and represents the final barrier to albumin entering the urinary space [76]. The foot processes of podocytes, in DN, broaden and efface, and there is a loss of podocyte-specific proteins such as nephrin and eventually loss of podocytes themselves. Such changes in podocytes may contribute to the development of albuminuria, a hallmark of DN.

Several lines of evidence showed that VEGF-A, the best characterized angiogenic factor, is a critical crosstalk protein among the three components of the glomerular filtration barrier, which is, a fenestrated endothelium, a 300-350 nm thick glomerular basement membrane, and the podocyte [77]. VEGF is a member of a family of secreted 34 – 42 kDa dimeric glycoproteins related to the platelet-derived growth factor family. As homodimeric glycoproteins, VEGF bind to two receptors: VEGFR1 (Flt-1) and VEGFR2 (Flk-1). It is highly expressed in podocytes, distal tubules, collecting ducts, and to a lower degree, proximal tubules [78]. In DN, mesangial cells transform into a pro-sclerotic phenotype and secrete VEGF in response to several factors relevant to diabetes, including TGF- $\beta$ , AGEs, and angiotensin II [79,80]. Moreover, the overproduction of ROS by podocytes increases urinary protein excretion and podocyte injury and might contribute to the initiation and progression of DN.

The increased expression of VEGF in the diabetic kidney is associated with hyperfiltration, proteinuria and glomerular hypertrophy [81], and these conditions are suppressed by the blocking of VEGF [82]. An acute infusion of VEGF into experimental animals markedly increased permeability to albumin in the kidney and other tissues supports the important role of VEGF in the pathogenesis of proteinuria in the diabetic kidney [83]. Very recent study has also suggested that increased podocyte Vegf<sub>164</sub> (the most abundant Vegf isoform) signaling dramatically worsens DN in a STZ-induced mouse model of diabetes, resulting in nodular glomerulosclerosis and massive proteinuria [84]. Lee et al. [85] demonstrated that high glucose significantly increases intracellular ROS and upregulates VEGF mRNA and protein expression in podocytes. They also shown that antioxidants inhibit high-glucose- and PMA-induced VEGF expression and that inhibition of PKC by a PKC- $\beta$  inhibitor, hispidin, or by a specific PKC inhibitor, GF109203X, also suppresses high-glucose- and PMA-induced VEGF expression in podocytes. They suggested that ROS are signaling molecules that are both downstream and upstream of PKC, and provide signal amplification in high-glucose-induced VEGF expression by podocytes [85]. In primary cultured rat mesangial cells, exposure to high glucose significantly increased VEGF mRNA, which is blocked by antioxidant, Tempol, and antisense oligonucleotides directed against p22phox, a NADPH subunit. Furthermore, high glucose-induced VEGF mRNA was also prevented by PKC- $\beta$ <sub>1</sub> inhibitor, suggesting a possible connection between VEGF and ROS as well as PKC [86].

## Role of ROS-PKC in ECM accumulation

In diabetes, the expression and accumulation of ECM protein are regulated by peptide growth factors, such as platelet-derived

growth factor, angiotensin II, and TGF- $\beta_1$  [87-89]. Of these, TGF- $\beta_1$ , a major mediator of the hypertrophic and pro-sclerotic changes in diabetic kidney disease, contributes to glomerular ECM accumulation by increasing the expression of ECM genes, such as collagen I and IV, and fibronectin and by decreasing ECM degradation through elevated expression of plasminogen activator inhibitor-1 (PAI-1) [90]. Moreover, TGF- $\beta_1$  promotes cell-matrix interactions by upregulating integrins, the cell surface receptors for matrix [91]. TGF- $\beta_1$  expression and activation play a pathogenic role in mesangial expansion in DN. High-glucose concentration upregulates the expression and bioactivity of TGF- $\beta_1$  in mesangial cells [92], in renal cortical fibroblasts [93], and in renal proximal tubules [94], suggesting that almost all renal cell types are involved by high ambient glucose.

In animal studies, the development of diabetic renal disease is likely caused by the increased activity of the renal TGF- $\beta$  system. Antagonism of TGF- $\beta$  by neutralizing monoclonal antibodies in STZ-induced diabetic mice prevented glomerular hypertrophy and attenuated the increase TGF- $\beta_1$ ,  $\alpha 1(IV)$  collagen, and mRNA fibronectin [95]. In addition, treatment with monoclonal anti-TGF- $\beta_1$  antibody in db/db mice, prevented the mesangial matrix expansion and preserved the creatinine clearance [96]. These studies strongly support the hypothesis that overactivity of the TGF- $\beta$  in the kidney is a crucial mediator of diabetic renal hypertrophy and mesangial matrix expansion. Ha et al. [97] has shown that high glucose-induce ROS, thus activated signal transduction cascade (PKC, MAPK, and janus kinase/signal transducers) and transcription factors (NF- $\kappa$ B, activated protein-1), up-regulate TGF- $\beta_1$ , angiotensin II (Ang II), and monocyte chemoattractant protein-1 (MCP-1) gene and protein expression and promote formation of AGE. In turn, PKC, TGF- $\beta_1$ , Ang II, and AGE also induce cellular ROS and signal through ROS leading to enhanced ECM synthesis [97].

### Inflammation in diabetic nephropathy

The pathogenesis of DM is characterized by activation of multiple molecular pathways, and accumulating evidence now suggests that inflammation have a central role in the development of diabetic complications, including DN. It has been proposed that the inter-link between the inflammatory process and development of DN involves complex molecular network processes. The core importance of inflammation is clearly evident in diabetic animals, where inflammation is driven by the abnormal metabolism and renal hypoxia [98]. Moreover, studies have reported a close relation between renal inflammation and the severity of metabolic and hypoxic disturbances in animals with DN, and demonstrated that decreasing systemic and renal neutrophils limit renal injury in experimental DN [98,99]. Surprisingly, the inflammatory-fibrotic features in human DN were strikingly similar to those documented in animal studies [98,100]. Infiltration of macrophages into the glomeruli and interstitium is one of the characteristic features of DN in addition to mesangial matrix expansion and interstitial fibrosis. Previous study has demonstrated that the number of macrophages in the glomeruli was significantly higher in moderate-stage than advanced-stage diabetic glomerulosclerosis [101]. It has also been shown that glomerular and interstitial injury is associated with macrophage infiltration in type 1 and 2 diabetes [102,103]. Persson et al. [104] have shown that the inflammation and endothelial dysfunction could well act as a predictor for the development of DN in irbesartan treated patients with type

2 DM [104]. The inflammatory response has been found higher in the diabetic patients than the non-diabetic patients [105]. From the above results, it is suggested that inflammation has emerged as a key pathophysiological mechanism. The various molecules are involved in the highly complex processes, i.e. the chemokines and their receptors, adhesion molecules, transcription factors and inflammatory cytokines [26].

### Chemokines

Migration of immune cells into the renal cell is a feature of early DN. Numerous studies have suggested that the monocytes, macrophages, lymphocytes and neutrophils are playing a critical role for the renal vascular damage through the variety of mechanisms, such as production of ROS, cytokines and proteases which eventually leads to renal vascular sclerosis [103]. Furthermore, the elevated level of macrophages from the T-cell greatly impairs the level of proteinuria in diabetic patients [105]. Monocytes are attracted by the specific chemokines to the place of organ damage, i.e. MCP-1. It has been reported that the accumulation and activation of MCP-1 is a critical determinant for the development of DN mainly by involving in the process of recruitment of macrophage, albumin excretion level, and tubulointerstitial damage [106]. Recently, we also have found the increased MCP-1 protein expression in STZ-induced kidney disease [107]. In addition, CX3CL1 is another chemokine and has been reported to play a role in the development of renal injury with its receptor CX3CR1. Its primary role is to act as chemoattractant for monocytes, T-cells and natural killer cells and various factors has been reported to activate this chemokine, such as elevated glucose level, AGEs and the activation of cytokine through various processes, including the activation of NF- $\kappa$ B and p38 MAPK-dependent and independent pathway [108,109].

### Adhesion molecules

Adhesion molecules are cell-surface protein, critically involving in the activation of leucocytes and macrophages to the site of inflammation as well as to the attachment of endothelium [110]. It includes intracellular adhesion molecule protein-1 (ICAM-1), vascular cell adhesion protein-1 (VCAM-1), endothelial cell-selective adhesion molecule, E-selectin and  $\alpha$ -Actinin 4. Of these ICAM-1 and VCAM-1 have been elaborately studied and have been reported to be up-regulated in diabetic animal models. ICAM-1 primarily involve in the migration of T-cells into the kidney and critically participate in the development of DN with the development of renal hypertrophy, mesangial matrix expansion and albuminuria. It has been found that the various ICAM-1 knockout mice animal models have been shown to ameliorate the progression of DN [111]. Furthermore, the inhibition of ICAM-1 expression and macrophage infiltration by anti-inflammatory agents could ameliorate experimental DN [112,113]. Several possible mechanism of ICAM-1 induction in diabetic renal tissue have been proposed, namely, induction of ICAM-1 by inflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1, interferon- $\gamma$ , and activation of PKC [114], AGEs enhance the expression of cell adhesion molecules [115], shear stress [116], oxidative stress [117], and osmotic agents [118]. Recent study has demonstrated that ERK, p38 MAPK, and JNK signaling pathways are involved in the expression of ICAM-1 induced by both high glucose and high osmolarity in human glomerular endothelial cells [119]. VCAM-1 is primarily involved in the adhesion of lymphocytes, monocytes, basophils and neutrophils and it also has been reported to be upregulated in diabetic patients

[120]. Previous study has demonstrated that chronic inhibition of p38 MAPK reduced ICAM-1 and VCAM-1 expression in the quadriceps muscle in diabetic rats [121], which suggest that MAPKs pathway play a significant role in inflammatory process in DN.

### Proinflammatory cytokines

It has demonstrated that renal cells, such as glomerular, endothelial, mesangial and tubular epithelial cells are able to synthesize the proinflammatory cytokines such as IL-1, 6 and 18 and TNF- $\alpha$  during the high glucose condition [102]. Several reports have suggested that stimulation of IL-1 could potentially activate various renal cells and eventually stimulate the synthesis of VCAM-1 and ICAM-1 [122]. Furthermore, it has been found to be a potential mediator for the intraglomerular abnormalities apart from its involvement in the vascular permeability as well as in matrix synthesis. Similarly, IL-6 has been reported to be associated with the increased renal hypertrophy as well as renal fibrosis [123]. It has been reported that IL-6 has a strong relation with enhancement of glomerular basement thickening and structural abnormalities in patients with type 2 DM [124]. The overexpression of IL-6 is also related to increased rates of urinary albumin excretion [125]. IL-18, a proinflammatory cytokines that belongs to the IL-1 superfamily, has been shown to play a significant role in the progression of DN. Study has shown that, in patient with DN, serum level of IL-18 as well as urinary excretion of this cytokine were elevated. These alterations were independent and strongly related to markers of glomerular and tubulointerstitial injury [126,127]. TNF- $\alpha$  has also been demonstrated to play most crucial role in renal hypertrophy as well as renal alternations during the early stage of DN [128]. Numerous studies have provided the direct evidence that the mRNA expression of TNF- $\alpha$  was elevated in diabetic patients, in comparison to non-diabetic patients [128,129]. High glucose-induced increased TNF- $\alpha$  level is associated with a toxic result to the renal cells and cause disturbance to the renal filtration rate by causing the hemodynamic disproportion between the vasodilation and vasoconstriction mediators [130]. Furthermore, it has been found to induce the ROS in mesangial cells and has been implicated to activate the NADPH oxidase subunits through PKC as well as MAPK pathways [131]. Considering all these reports, protection of the progression of DN in patients with high blood glucose level seems to be challenging and the chemical moiety which effectively control the stimulation of potential cytokine, such as IL-1,6, 18 and TNF- $\alpha$  could play a major for the protection against DN.

### Conclusion

Excess amounts of glucose induced the activation of PKC-MAPK in association with increased ROS, and inflammatory signaling cascades and as a result the activation of various cytokines and transcription factors which eventually cause increased expression of ECM genes with the progression to fibrosis and end stage renal disease. Understanding the interactions among these important signaling pathways is crucial for blocking the progression of DN. Identifying new therapeutic targets and treatments that could potentially affect the primary mechanisms that contribute to the pathogenesis of DN is of great clinical importance. Better understanding of the role of PKC-MAPK, oxidative stress, and inflammatory processes will assist the development of new and effective therapeutic targets which can be extrapolated into clinical applications both for preventing and for halting the progression of DN.

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