Diabetes mellitus is known to trigger retinopathy, neuropathy and nephropathy. The cellular elements of the kidney, i.e., glomerular endothelia, mesangial cells, podocytes, and tubular epithelia, are targets for hyperglycemic injury. Glomerulosclerosis, thickening of the glomerular basement membrane, glomerular hypertrophy, mesangial cell expansion, podocyte loss, renal-cell hypertrophy and tubulointerstitial fibrosis are among the major pathological changes that occur during the course of diabetic nephropathy, which ultimately results in progressive albuminuria, reduction in glomerular filtration rate, elevation of arterial blood pressure and fluid retention (Mauer et al., 1984; Ziyadeh, 1993; Dalla Vestra et al., 2000; Mason and Wahab, 2003). Numerous efforts have been made to identify the major causative agents in the pathogenesis of this disease, and the data suggest that among the numerous pathways activated during the course of the disease are the following: a hemodynamic pathway, involving the renin-angiotensin-aldosterone and urotensin systems; profibrotic and inflammatory cytokines, including TGF-β and tumor necrosis factor-α (TNF-α); various kinases such as protein kinase C (PKC) and the Janus kinase pathway; and, most importantly, oxidative stress mediators, such as nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase). However, clinical strategies based on these pathways for the management of diabetic nephropathy remain unsatisfactory, as the number of diabetic patients with nephropathy is increasing yearly. To develop ground-breaking therapeutic options to prevent the development and progression of diabetic nephropathy, a comprehensive understanding of the molecular mechanisms involved in the pathogenesis of this disease is mandatory. The complex molecular mechanism involved in the pathogenesis of diabetic nephropathy has been depicted in Figure 1.
Role of Renin Angiotensin Aldosterone System in diabetic nephropathy

The renin-angiotensin aldosterone system (RAAS) plays an integral role in the homeostatic control of arterial pressure, tissue perfusion, and extracellular volume. In addition to its systemic and local renal hemodynamic effects, RAAS also influences renal tissue cell infiltration and inflammation (Fujihara et al., 2000; Benigni et al., 2004; Graciano et al., 2004; Franco et al., 2007). Thus, the dysregulation of RAAS may lead to hypertension and renal tissue injury.

Role of Renin in diabetic nephropathy

A growing body of evidence suggests that renin and its receptor [(Pro)renin receptor (PRR)] play a pivotal role in the pathogenesis of diabetic nephropathy. It has also been noted that PRR is involved in the development and progression of kidney disease during diabetes by enhancing the renal production of inflammatory cytokines, such as TNF-α and IL-1β, independent of the effects of renal angiotensin II (Ang II) (Matavelli et al., 2010). In addition, renin itself regulates the expression of TGF-β1 in mesangial cells through a receptor-mediated mechanism that in turn stimulates PAI-1, fibronectin and collagen I (Huang et al., 2006; Zhang et al., 2008). Further, another study noted that renin in the culture medium upregulated the expression levels of TGF-β1, type IV collagen and VEGF, each of which was abolished by treatment with aliskiren, a renin inhibitor, confirming the detrimental role of renin in the induction and progression of diabetic nephropathy (Kang et al., 2011). Interestingly, prior treatment with aliskiren under high glucose conditions also significantly decreased the expression of TGF-β1, type IV collagen, VEGF and PAI-1 (Kang et al., 2011). Taken together, it may be concluded that renin and its receptor are
involved in the pathogenesis of diabetic nephropathy mainly by enhancing the renal production of fibrotic elements and inflammatory cytokines (Figure 2); additionally, the renoprotective potential of aliskiren was confirmed by its direct anti-fibrotic effects.

**Role of Angiotensin II in diabetic nephropathy**

Ang II is the most powerful biologically active product of the RAAS. Over-activation of intrarenal Ang II leads to the development of hypertension and renal injury and results in reduced renal function and structural changes in the kidney (Navar et al., 2003; Navar, 2005). In addition, Ang II directly induces podocyte injury via the activation of Ang II receptor type 1 (AT-1) receptors, independent of hemodynamic changes (Durvasula et al., 2004; Liang et al., 2006; Liebau et al., 2006). Moreover, Ang II interacts with various local autocrine and paracrine factors, such as nitric oxide (NO), eicosanoids, adenosine, and superoxide, to influence the glomerular filtration rate (Granger and Schnackenberg, 2000). It is of interest to note that glucose increases the expression of the angiotensinogen gene in proximal tubule cells and Ang II production in mesangial cells, suggesting that high glucose itself activates the renin-angiotensin system (Singh et al., 1999; Leehey et al., 2000; Vidotti et al., 2004). Numerous studies suggest that Ang II has been implicated in the progression of diabetic nephropathy through multiple pathways (Figure 2). Ang II increases the level of mesangial TGF-β mRNA and increases the production of both latent and active TGF-β, which in turn results in extra cellular matrix (ECM) accumulation by increasing the synthesis of matrix proteins, such as fibronectin, collagens, and laminin, and by inhibiting matrix degradation (Kagami et al., 1994; Jssus Egido, 1996). This was confirmed by another study in which Ang II antagonism
was shown to exert a renoprotective effect through its negative influence on TGF-β (Junaid et al., 1997). It has also been noted that TGF-β further increases the expression of PAI-1 (Kutz et al., 2001). It is still of interest to note that Ang II itself directly increases PAI-1 expression through an AT₁ receptor-dependent mechanism independent of TGF-β (Kagami et al., 1994; Kagami et al., 1997; Nakamura et al., 2000). Apart from fibrotic elements, such as TGF-β & PAI-1, Ang II has also been shown to cause renal fibrosis by upregulating the expression of Rho-A and activating the Rho/Rho kinase pathway (Ruiz-Ortega et al., 2006). Further, such inflammatory cytokines as IL-6 and monocyte chemoattractant protein-1 (MCP-1) have been noted to be involved in the development and progression of diabetic nephropathy (Wada et al., 2000; Navarro and Mora-Fernandez, 2008). Interestingly, Ang II regulates the expression of inflammatory cytokines, such as IL-6 and MCP-1, in the kidney (Kato et al., 1999; Navarro and Mora-Fernandez, 2008). The detrimental role of Ang II was confirmed when it was shown that the inhibition of Ang II improves diabetic nephropathy through the suppression of renal MCP-1 (Amann et al., 2003). Moreover, VEGF has been implicated in the pathogenesis of diabetic nephropathy, as VEGF is upregulated early in diabetes mellitus, especially in podocytes (Cooper et al., 1999; Cha et al., 2000; Cha et al., 2004). Ang II stimulates the synthesis of VEGF in podocytes through the activation of the p38 mitogen-activated protein kinase (p38 MAPK) pathway, suggesting another mechanism through which Ang II may play a crucial role in the pathogenesis of diabetic nephropathy (Kang et al., 2006). Numerous studies have also revealed that Ang II increases the renal production of ROS by activating NADPH oxidase (Giacchetti et al., 2005). Ang II also mediates ROS generation via the activation of NADPH, which in turn further stimulates VEGF.
synthesis (Feliers et al., 2006). Taken together, it may be concluded that Ang II is a master molecule of renal injury during diabetes.

**Role of Aldosterone in diabetic nephropathy**

Aldosterone has been considered one of the major mediators in the pathogenesis of diabetic nephropathy (Fujisawa et al., 2004; Guo et al., 2006; Han et al., 2006), and the blockade of mineralocorticoid receptors (MCR) was shown to prevent the development and progression of diabetic nephropathy (Guo et al., 2006; Han et al., 2006a; Taira et al., 2008). Interestingly, hyperglycemia has been shown to increase renal aldosterone levels by inducing CYP11B2 expression (Xue and Siragy, 2005; Taira et al., 2008; Siragy and Xue, 2008). In the kidney, mesangial cells are known to produce aldosterone in response to Ang II, which results in ECM accumulation (Lai et al., 2004). Moreover, a recent study demonstrated that a local aldosterone system is involved in podocyte injury under diabetic conditions (Lee et al., 2009). However, among the components of RAAS, Ang II has received the greatest consideration as a mediator of renal injury during diabetes, and the pathological role of Ang II in the progression of diabetic nephropathy has been extensively discussed. Despite this, numerous studies have revealed that aldosterone is also a potentially important component of the RAAS and may be involved in the induction and progression of diabetic nephropathy. In experimental diabetic nephropathy, treatment with spironolactone, an aldosterone receptor antagonist, has been shown to decrease albuminuria and mitigate glomerulosclerosis by downregulating the renal expression of matrix-regulating genes, such as TGF-β, MMP, VEGF and insulin growth factor (Han et al., 2006a). Further, spironolactone was noted in a clinical study to provide renoprotective effects by reducing oxidative...
stress and attenuating the overexpression of MCP-1 in patients with diabetic nephropathy (Takebayashi *et al.*, 2006). Together, these studies demonstrate the direct detrimental role of aldosterone in the pathogenesis of diabetic nephropathy (Figure 2). Aldosterone has been noted to induce renal injury through its effects on PAI-1 expression (Brown *et al.*, 2000). Further, the renal production of aldosterone induces inflammation and matrix formation in the kidneys of diabetic rats (Cha *et al.*, 2005; Siragy and Xue, 2008). In addition, aldosterone accelerates renal damage by inducing the production of growth factors and ROS and interfering in the process of extracellular matrix degradation (Remmuzzi *et al.*, 2008). Taken together, it may be suggested that aldosterone antagonism may provide a prolific therapeutic strategy in the management of nephropathy in diabetic patients.

**Role of Protein kinase C in diabetic nephropathy**

PKC, a family of serine threonine kinases that consists of at least 15 isoforms, including PKC-α, -β₁, -β₂, -δ and -ε, has been documented to be activated in the glomeruli of diabetic rats, as well as in mesangial cells exposed to high glucose (Koya *et al.*, 1997; Babazono *et al.*, 1998; Whiteside and Dlugosz, 2002). PKC activation is involved in regulating a number of vascular functions, such as contractility, cell proliferation and extracellular matrix protein synthesis (Rasmussen *et al.*, 1984; Kariya *et al.*, 1987; Huhtala and Tryggvason, 1990). The PKC-α isoform has been noted to be implicated in the pathogenesis of diabetic nephropathy by upregulating VEGF expression (Yao *et al.*, 2006). Apart from PKC-α, other isoforms of PKC, such as PKC-β and PKC-ε, have also been documented to mediate high glucose-induced VEGF expression in mesangial cells (Xia *et al.*, 2007). Further, a study using PKC-β null mice (lacking PKC-β) confirmed that PKC-β activation induces renal dysfunction.
by increasing the expression of Nox-2, Nox-4, endothelin-1, VEGF, TGF-β, and CTGF as well as oxidant production (Ohshiro et al., 2006). Previously, it was reported that the diabetes-induced activation of the PKC-β isoform may play a detrimental role in the pathogenesis of diabetic nephropathy by inducing renal fibrosis through the upregulation of TGF-β, type IV collagen, laminin and fibronectin in the glomeruli of diabetic rats (Koya et al., 1997). In another study, the PKC-β isoform was noted to be activated in the glomeruli of diabetic db/db mice, and treatment with a PKC-β-specific inhibitor not only inhibited glomerular PKC activation but also ameliorated an increase in urinary albumin excretion. This treatment further restored the structural changes, such as mesangial expansion, through a reduction in the glomerular expression of TGF-β and in ECM accumulation (Koya et al., 2000). Moreover, hyperglycemia-induced PKC-δ activation has been noted to increase extracellular matrix production by enhancing responsiveness to TGF-β in mesangial cells (Hayashida and Schnaper, 2004). However, it is interesting to note that, while investigating the role of PKC isoforms using different PKC isoform-specific knock-out mice, the involvement of activated PKC-α was associated with the occurrence of albuminuria during the course of diabetic nephropathy. Diabetic PKC-α knock-out mice were protected from albuminuria, but renal and glomerular hypertrophy were not prevented because the expression of TGF-β was not reduced (Menne et al., 2004). In contrast, PKC-β plays a critical role in diabetes-induced tubular hypertrophy and glomerular enlargement; PKC-β knock-out mice were protected against the development of renal and glomerular hypertrophy, as well as from mesangial expansion by a reduction in TGF-β, CTGF and matrix molecule expression under diabetic conditions. Still, albuminuria was not prevented in PKC-β knock-out mice (Ohshiro et al., 2006; Meier et al., 2007). However, during a clinical investigation, it
was noted that ruboxistaurin, a selective PKC-β inhibitor, provided renoprotective effects by reducing albuminuria and maintaining eGFR over 1 year in type 2 diabetic patients with nephropathy (Tuttle et al., 2005). Further, the activation of PKC-α and PKC-β isoforms was noted to be associated with increased NADPH activity and NADPH-dependent superoxide production, suggesting a common pathway among these PKC isoforms in the induction of renal injury (Ohshiro et al., 2006; Thallas-Bonke et al., 2008). Taken together, these studies demonstrate that the mechanism involving these PKC isoforms in the induction and progression of diabetic nephropathy seems to be very complex (Figure 3). Therefore, it may be the case that the proper selection of selective inhibitors of PKC isoforms plays a crucial role in the management of diabetic nephropathy.

**Role of NADPH oxidase in diabetic nephropathy**

NADPH oxidase is a multimolecular enzyme consisting of two membrane-bound elements, the α-subunit (gp91^PHOX^) and β-subunit (p22^PHOX^), three cytosolic components (p67^PHOX^, p47^PHOX^ and p40^PHOX^), and a low-molecular-weight G protein (either rac 2 or rac 1). Among these components, gp91^PHOX^ is considered to be an essential element, as electron carrying components are bound to it; p67^PHOX^ is generally involved in the transfer of electrons directly from NADPH to oxygen to form superoxide; p47^PHOX^ carries the cytosolic proteins to the membrane proteins to assemble the active oxidase; and p22^PHOX^ has a tail in the cytosol that binds to p47^PHOX^ (phosphorylated p47^PHOX^) and brings the entire cytosolic oxidase complex to the membrane to assemble the active oxidase. Interestingly, mesangial cells express all of the components required for functional NADPH oxidase systems, except for the catalytic subunit gp91phox (Jones et al., 1995). However, homologues of gp91phox
were identified, and Nox-2 expression was found in podocytes, mesangial cells, and endothelium; Nox3 was found in the fetal kidney; and Nox-4 was found in the glomerulus, proximal tubule, and distal convoluted tubule (Babior, 2004; Gill and Wilcox, 2006; Sedeek et al., 2009). Nox-4 (originally called renal NADPH oxidase or Renox) was reported to be most abundantly expressed in the kidney as a renal source of ROS production (Geiszt et al., 2000; Shiose et al., 2001; Sedeek et al., 2009), and Rac was shown to play a crucial role as a modulatory protein for Nox-4 in renal mesangial cells (Gorin et al., 2003). At the membrane, these proteins assemble into a gp91phox-p22phox heterodimer and induce a conformational change in gp91phox, which results in superoxide production (Li and Shah, 2003). High glucose concentrations increase the expression of the NADPH oxidase subunits p22phox and p47phox in mesangial cells in vitro and in vivo in a PKC-dependent manner (Etoh et al., 2003; Gorin et al., 2005; Xia et al., 2006). Further, high glucose induces intracellular ROS in mesangial cells and tubular epithelial cells, which can be effectively blocked by the inhibition of NADPH oxidase; this suggests the pivotal role of NADPH oxidase in high glucose-induced ROS generation (Ha and Lee, 2000; Lee et al., 2003). It is interesting to note that NADPH oxidase-mediated ROS further stimulates additional enzyme systems, e.g., ROS-dependent NOS uncoupling, particularly of eNOS, whereby the enzyme no longer generates NO but rather becomes a source of superoxides (Vasquez-Vivar et al., 1998; Vasquez-Vivar and Kalyanaraman, 2000). NADPH oxidase-mediated renal oxidative stress promotes mesangial expansion and albuminuria by increasing the expression of fibronectin and collagen-1 in the kidney (Asaba et al., 1998). In addition, NADPH oxidase-derived ROS has been noted to be involved in methylglyoxal (MGO)-induced renal fibrosis, advanced oxidation protein products (AOPPs)-induced inflammation in the kidney.
and direct oxidative damage to the DNA of diabetic glomeruli (Kitada et al., 2003; Etoh et al., 2003). The detrimental role of NADPH oxidase in the development of diabetic nephropathy has been further confirmed by numerous studies in which treatment with NADPH oxidase inhibitors markedly attenuated the progression of nephropathy by reducing the occurrence of albuminuria and preventing the development of glomerulosclerosis through a reduction of renal oxidative stress (Thallas-Bonke et al., 2008; Winiarska et al., 2008; Nam et al., 2009; Ribaldo et al., 2009). In addition, the inhibition of Nox-4 oxidase by the administration of ‘AS Nox-4’, a phosphorothioated antisense NOX, reduced whole kidney and glomerular hypertrophy in the diabetic cortex and glomeruli. This suggests the direct involvement of Nox-4 in renal hypertrophy, as well as a mechanism involving Nox-4-derived ROS mediated Akt/PKB and ERK1/2 activation that increases fibronectin expression in the kidney (Groin et al., 2005). Further, Nox-4-derived ROS generation was noted to be involved in Ang II-induced protein synthesis in mesangial cells and renal hypertrophy (Groin et al., 2004; Tabet et al., 2008). Moreover, Nox- based NADPH oxidase has been noted to regulate the growth and profibrotic processes of renal cells through redox-sensitive p38 MAPK, which suggests that renal oxidative stress and profibrotic signaling pathways in diabetic conditions are mediated via the increased expression/activation of cortex-specific Nox4-based NADPH oxidase (Seedek et al., 2010). More recently, TNF-α (a nuclear factor kappa B (NF-κB)-induced tumor necrosis factor)-inducible protein 8 (TNFAIP8), a recently identified protein, has been shown to be a critical component in mesangial cell proliferation during diabetic renal injury. Interestingly, NADPH oxidase has been shown to regulate the renal expression of TNFAIP8 (Zhang et al., 2010). Thus, through increased elucidation of the key interactions between NADPH oxidase and the critical pathways implicated in the
progression of diabetic nephropathy, it appears that this enzyme is likely to be an appropriate therapeutic target for further investigation in human diabetic nephropathy.

**Role of Transforming Growth Factor-β in diabetic nephropathy**

The TGF-β superfamily is composed of many multifunctional cytokines, including TGF-βs, activins, inhibins, anti-mullerian hormone, bone morphogenetic proteins, and myostatin (Piek et al., 1999). Previously, it was generally considered that the effects of the three TGF-β isoforms (TGF-β1, TGF-β2 & TGF-β3) on matrix production were similar when added to cells in vitro (Graycar et al., 1989); however, TGF-β1 seems to be the main "fibrogenic" cytokine in vivo (Zugmaier et al., 1991; Terrell et al., 1993). More recently, it was demonstrated that the overexpression of TGF-β isoforms may occur in different patterns in different tissues. For example, during acute injury, compared to chronic fibrosis in normal kidney, the weak expression of different isoforms of TGF-β mRNA in a few glomerular cells follows a TGF-β1>TGF-β3>>TGF-β2 pattern; however, no remarkable difference was found in the levels of mRNA expression in the glomeruli and the tubulointerstitium during diseased states (Yamamoto et al., 1996). It is worth noting that the TGF-β isoforms play a crucial role in the induction of renal fibrosis through the regulation of matrix accumulation markers. The glomerular and tubulointerstitial depositions of fibronectin were only correlated with TGF-β1. PAI-1 was correlated with TGF-β1 and TGF-β3, but not with TGF-β2 (Yamamoto et al., 1996), which suggests a more direct relationship between TGF-β1 expression and the deposition of matrix proteins. However, a different study suggested that the levels of TGF-β2 elevate rapidly in glomeruli and tubules during the acute phase of experimental diabetes (days 3–14),
Chapter 2

Review of literature

when the rate of synthesis of collagen-I increased maximally in the rat kidney cortex. Treatment with TGF-β2 antibody has been shown to have renoprotective effects during the first 14 days of diabetes by attenuating the synthesis of collagen-I and urinary albumin (Hill et al., 2000; Hill et al., 2001), suggesting a role for TGF-β2 in the acute pre-fibrotic stages of diabetic nephropathy and a contributory role for these isoforms in the induction of renal fibrosis. Further, these findings also confirm that the overexpression of TGF-β isoforms may occur in different patterns during acute injury, compared to chronic fibrosis. In addition, clinical studies have demonstrated that all three isoforms of TGF-β were found to be elevated in both the glomerular and tubulointerstitial compartments of patients with diabetic nephropathy (Yoshioka et al., 1993; Yamamoto et al., 1993). Moreover, glomerular TGF-β1 mRNA was noted to be markedly increased in renal biopsy specimens from patients with proven diabetic kidney disease (Iwano et al., 1996). TGF-β1 is secreted in a latent complex and activated in mesangial cells by high glucose-induced thrombospondin expression (Poczatek et al., 2000). Increased expression of TGF-β has been implicated in the pathogenesis of diabetic nephropathy by increasing PAI-1 synthesis; stimulating fibronectin synthesis in mesangial cells; decreasing the activity of PA and MMP-2; stimulating the expression of CTGF, which promotes the renal deposition of extracellular matrix components such as collagen I & IV; stimulating VEGF expression; and enhancing the expression of IL-18 in human renal proximal tubular epithelial cells. TGF-β exerts its renal fibrotic action mainly through its downstream signaling molecules Smad2 and Smad3, which, interestingly, are negatively regulated by the inhibitory Smad7, which inhibits renal fibrosis and inflammation (Schiffer et al., 2002; Isono et al., 2002; Wang et al., 2005; Lan and Chung, 2011). It is
worthwhile to note that Smad2 and Smad3 are strongly activated in both experimental and human kidney diseases, including diabetic nephropathy (Isono et al., 2002; Fujimoto et al., 2003; Li et al., 2004). However, Smad7 expression is lost in the diabetic kidney, which causes an imbalance within the TGF-β/Smad signaling pathways and results in the development of renal fibrosis (Chen et al., 2010). The TGF-β/Smad signaling pathways involve α2(I)-collagen gene induction in human mesangial cells to have renal fibrogenic properties (Poncelet et al., 1999). The detrimental role of TGF-β/Smad signaling pathways were further confirmed when treatment with anti-TGF-β antibody was shown to significantly reduce renal fibrosis and proteinuria through the inhibition of Smad/TGF-β signaling (Fukasawa et al., 2004). In addition to the TGF-β/Smad2/3 signaling pathway, TGF-β has been implicated in fibrosis by the activation of MAPK signaling pathways, including c-Jun N-terminal kinase, extracellular-regulated kinase, and p38 MAPK (Atfi et al., 1997; Choi, 2000; Chin et al., 2001). This suggests that TGF-β activates various downstream signaling pathways that collectively play an important role in the pathogenesis of diabetic nephropathy (Figure 4). In addition to regulating these fibrotic elements, TGF-β also upregulates the expression of GLUT-1 in mesangial cells (Inoki et al., 2000), which increases the concentration of intracellular glucose and accelerates the progression of metabolic abnormalities in the diabetic kidney (Haneda et al., 2003). Moreover, TGF-β has also been noted to be involved in the NADPH oxidase-mediated generation of ROS in mesangial cells exposed to high glucose (Tojo et al., 2007). Thus, targeting TGF-β-activated signaling pathways in the diabetic kidney may provide new strategies to prevent or treat diabetic renal injury.
Chapter 2  Review of literature

Role of Tumor Necrosis Factor-α in diabetic nephropathy

Inflammation plays a pivotal role in the pathogenesis of diabetic nephropathy. Inflammatory cytokines, primarily IL-1, IL-6, IL-18, and TNF-α, are involved in the development and progression of diabetic nephropathy (Hasegawa et al., 1991; Sekizuka et al., 1994; Navarro et al., 1999; Moriwaki et al., 2003). TNF-α is one of the main proinflammatory cytokines and may be produced intrinsically in renal cells (Nakamura et al., 1993; Ortiz and Egido, 1995). TNF-α expression levels have been observed in the tubulointerstitium as well in proximal tubular epithelial cells, and elevated levels of TNF-α have been noted to play a crucial role in renal injury by the reduction of blood flow and filtration rate and the alteration of the barrier function of capillary walls (Baud and Ardaillou, 1995). Interestingly, TNF-α mRNA and protein levels were found to be markedly increased in the kidneys of diabetic rats compared with kidneys from normal rats (Nakamura et al., 1993; Sugimoto et al., 1999; Navarro et al., 2005). Nephrin is an important regulator of the glomerular filtration barrier, and its malfunction is associated with severe proteinuria. Interestingly, TNF-α was noted to upregulate nephrin expression in human embryonic kidney epithelial cells and podocytes, initiating the mechanism through which TNF-α promotes functional changes in the kidney (Huwiler et al., 2003). Further, because TNF-α mRNA is abundantly expressed in diabetic rats, it has been suggested that TNF-α may contribute to the induction of nephropathy by stimulating albuminuria (Kalantarinia et al., 2003). TNF-α mediates its biological effects by binding to two specific receptors: TNF receptor type 1 and TNF receptor type 2 (TNFR1 & TNFR2). It is worth noting that elevated concentrations of circulating TNFRs in patients with type 2 diabetes have been associated with progression to end-stage renal failure.
(Aggarwal, 2003; Hehlgans and Pfeffer, 2005; Niewczas et al., 2011). TNF-α has been shown to stimulate the release of other chemokines and growth factors, including MCP-1 and TFG-β (Vassalli, 1992; Feldmann et al., 1998), and to cause a reduction in glomerular PA (Colucci et al., 1993), suggesting the involvement of TNF-α in renal fibrosis. Treatment with pentoxifylline, a TNF-α synthesis inhibitor, was noted to possess anti-fibrotic potential in renal cells by inhibiting CTGF expression through interference with Smad3/4-dependent CTGF transcription, confirming the pathogenic role of TNF-α in diabetic nephropathy by the induction of renal fibrosis (Lin et al., 2005). The therapeutic potential of pentoxifylline was further confirmed by a meta-analysis involving 476 participants that demonstrated an association between TNF-α inhibition and a significant reduction in patients suffering from diabetic nephropathy (McCormick et al., 2008). Apart from the renal expression of TNF-α, urinary TNF-α excretion in diabetic patients has been associated with the severity of glomerular and tubulointerstitial damage in patients with type-2 diabetes mellitus (Navarro et al., 2006). In support of this view, it has been reported that treatment with infliximab, a monoclonal TNF-α antibody, markedly reduced urinary albumin excretion in diabetic rats (Moriwaki et al., 2007). TNF-α, NF-κB–TNFAIP8, a recently identified protein, has been noted to be a critical component in mesangial cell proliferation during diabetic renal injury (Zhang et al., 2010). In addition, it is worth noting that the renal production of TNF-α and IL-1β has been implicated in the PRR-mediated development of diabetic nephropathy, although PRR does not affect Ang II generation (Matavelli et al., 2010). Thus, further studies are required to evaluate the therapeutic potential of TNF-α inhibitors in the management of diabetic nephropathy.
Role of JAK/STAT pathway in diabetic nephropathy

Janus kinases (JAKs) are non-receptor tyrosine kinases consisting of four members: JAK-1, JAK-2, JAK-3, and TYK-2. These molecules activate the seven members of the signal transducers and activators of transcription (STATs), STAT-1, STAT-2, STAT-3, STAT-4, STAT-5a, STAT-5b, and STAT-6 by tyrosine and/or serine phosphorylation. Once the STATs have been activated, they transmit signals to cytosolic and nuclear targets, leading to alterations in cell growth, proliferation and other cellular functions (Darnell et al., 1994). Under high glucose conditions, JAK-2 and STAT-1, -3 and -5 were noted to be activated, along with TGF-β and fibronectin synthesis in glomerular mesangial cells. Interestingly, AG-490, a specific JAK-2 inhibitor, prevented this high glucose-induced increase in TGF-β and fibronectin synthesis by abolishing the tyrosine phosphorylations of JAK-2, STAT-1 and -3. This suggests that the activation of the JAK-2/STAT-1,-3 pathway by hyperglycemia plays an important role in promoting cell proliferation and the synthesis of ECM proteins in glomerular mesangial cells (Wang et al., 2002). Further, high glucose augments the Ang II-induced JAK/STAT pathway in glomerular mesangial cells by inducing the phosphorylation of JAK-2 kinase and different STAT proteins, namely STAT-1, STAT-3 and STAT-5a/b. These data suggest that these STATs may play an important role in glomerular mesangial cell growth and extracellular matrix deposition in diabetic nephropathy (Amiri et al., 2002). These studies support the earlier finding that the JAK-2/STAT-1/STAT-3 pathway was involved in advance glycation end products (AGE)-induced collagen production in NRK-49F cells (Huang et al., 1999), thereby identifying the JAK/STAT pathway as a critical mediator in renal fibrosis. The JAK/STAT pathways also play a crucial role in oxidative stress-mediated renal
injury through peroxynitrite, a highly reactive oxidant that promotes the structural and functional changes in the kidneys of diabetic rats through the activation of the JAK/STAT pathway (Wang et al., 2009). The detrimental role of the JAK/STAT pathway in the development of diabetic nephropathy has been further confirmed in a recent study using diabetic STAT-3 knockdown mice, which were shown to exhibit significantly less proteinuria, mesangial expansion, glomerular cell proliferation, and macrophage infiltration than diabetic reference mice (Lu et al., 2009). In addition, a reduction in STAT-3 activity abrogates the stimulation of inflammatory markers, including IL-6, intercellular adhesion molecule-1 (ICAM-1), MCP-1, activated NF-KB and type IV collagen, thus demonstrating that JAK2-STAT3 proteins may be involved in the early kidney damage associated with diabetes (Lu et al., 2009).

Interestingly, in a recent study, it was observed that in the tubulointerstitial compartment, some of JAK/STAT family members were downregulated in early stages of diabetic nephropathy, whereas most family members were expressed at higher levels in progressive diabetic nephropathy. On the other hand, in the glomerular compartment, these genes were highly expressed in early stages of diabetic nephropathy and downregulated in progressive diabetic nephropathy. Collectively, these results suggest that significant regulation of JAK-1, -2, and -3 and STAT-1 and -3 mRNAs in the glomeruli plays a crucial role in early as well as progressive diabetic nephropathy, although in the tubulointerstitium, these members are only involved in progressive diabetic nephropathy (Berthier et al., 2009). Thus, the JAK/STAT pathway seems to act as a central mediator in the pathogenesis of early and progressive diabetic nephropathy, and the inhibition of JAK/STAT may be a new strategy for the management of diabetic nephropathy.
Role of Adenosine in diabetic nephropathy

Adenosine is an autacoid that plays a crucial role in renal function, affecting such processes as the regulation of the glomerular filtration rate, renin release, intrarenal inflammation, and the growth of mesangial and vascular smooth muscle cells. Furthermore, it exerts physiological and pathophysiological actions depending upon its interactions with the following adenosine receptor (AR) subtypes: A1-AR, A2a-AR, A2b-AR and A3-AR (Lopez-Novoa et al., 1987; Olivera et al., 1989; Spielman and Arend, 1991; Vallon et al., 2006). Interestingly, the expression of all four adenosine receptors was found in the isolated kidneys of rats and mice (Dixon et al., 1996; Vitzthum et al., 2004). It is worth noting that diabetes mellitus induces A1-AR and A2a-AR mRNA expression and a subsequent increase in A1-AR and A2a-AR protein levels in the kidney (Pawelczyk et al., 2005). Conversely, the A2b-AR mRNA expression & protein content in the diabetic kidney were noted to be decreased. A3-AR mRNA levels in diabetic kidneys remained unchanged, but membrane-associated A3-AR protein levels increased (Pawelczyk et al., 2005). These changes in AR gene expression and receptor protein content signify the involvement of adenosine and its receptors in the pathogenesis of diabetes-induced nephropathy. The activation of A2a-AR has been noted to provide renoprotective effects during diabetic conditions, as the early administration of A2A agonists markedly reduces macrophage infiltration, inflammation, and functional and histological changes by decreasing the fibronectin mRNA and urinary MCP-1 associated with diabetic nephropathy (Awad et al., 2006). On the other hand, A2b-AR activation was noted to be involved in the high glucose-mediated induction of VEGF expression in kidney glomeruli, demonstrating a pathogenic condition similar to that observed in diabetic nephropathy (Valladares et
al., 2008). In addition, activation of A2b-AR mediates TGF-β1 release from the glomeruli of diabetic rats, further demonstrating the pathogenic events observed in diabetic nephropathy (Roa et al., 2009). Thus, further studies are required to investigate the ameliorative effect of selective A2a-AR agonists and A2b-AR antagonists, which may prove to be a novel class of compounds in the prevention and treatment of diabetic nephropathy.

Role of Cannabinoid Receptors in diabetic nephropathy

There are at least two types of cannabinoid receptors, including CB1 and CB2, that are coupled to G proteins and play a key role in controlling the peripheral energy metabolism. However, these receptors are expressed predominantly in the central nervous system. Interestingly, mRNA for both CB1 and CB2 receptors have also been found in mesangial cells (Deutsch et al., 1997). Elevated levels of renal CB1 receptors were noted to be involved in renal injury by the induction of renal hypertrophy, along with glomerular and tubulointerstitial lesions, ultimately resulting in increased proteinuria, plasma creatinine, and urea nitrogen levels in obesity-induced nephropathy (Janiak et al., 2007). The potential role of CB1 receptors in renal injury was further corroborated in another study, which demonstrated that the inhibition of CB1 receptors with AM281/ SR141716, CB1 receptor antagonists, attenuates cisplatin-induced increased p38 MAPK activation, oxidative/nitrosative stress, cell death and interrelated inflammatory cell infiltration in the kidney, resulting in decreased renal tubular cell death and a marked improvement in renal function (Mukhopadhyay et al., 2010). In addition, blocking CB1 receptor with AM251, a selective CB1 receptor antagonist, was noted to ameliorate albuminuria by preventing the downregulation of nephrin and podocin in diabetic mice; this confirms the
detrimental role of CB1 receptor activation in the pathogenesis of diabetic nephropathy (Barutta et al., 2010). Conversely, CB2 receptor activation limits the inflammation and interrelated oxidative/nitrosative stress cell death associated with cisplatin-induced nephropathy (Mukhopadhyay et al., 2010a). The role of oxidative stress and inflammation as the major detrimental players in the pathogenesis of diabetic nephropathy (Vasavada and Agarwal, 2005; Mora and Navarro, 2006) signifies the potential role of renal CB2 receptor activation in the management of diabetic nephropathy. This was confirmed when glomerular CB2 expression levels were shown to be reduced during diabetic nephropathy because of the prominent effect of CB2 down-regulation in podocytes (Barutta et al., 2011) and the activation of the CB2 receptor using selective CB2 agonists. These agonists provide renoprotective effects by significantly reducing albuminuria in diabetes-induced nephropathy in mice (Barutta et al., 2011). Overall, it has been observed that the upregulation of the CB1 receptor and downregulation of the CB2 receptor in the kidney play a crucial role in the pathogenesis of diabetic nephropathy. However, further clinical studies are needed to elucidate the protective effects of CB receptor modulators on renal function in patients with diabetes mellitus.
Role of other novel targets in diabetic nephropathy

Superoxide dismutase (SOD) is a major defender against excessive superoxide generation, and hyperglycemic conditions decrease SOD activity (Sindhu et al., 2004). Using SOD1-deficient C57BL/6-Akita mice, the deficiency of SOD1 was shown to be associated with increased renal mRNA expression of TGF-β1 and CTGF, reduced glomerular nitric oxide, and increased renal prostaglandin E₂ (PGE₂) production (Fujita et al., 2012). In addition to SOD, reduced expression of lipoic acid synthase has been noted to accelerate diabetic nephropathy by increasing microalbuminuria, glomerular basement thickening, and the mesangial matrix in diabetic mice (Yi et al., 2012), suggesting the direct renoprotective effect of SOD and lipoic acid synthase during diabetic conditions. Osteopontin (OPN) is a phosphoprotein produced by the kidney that mediates cell adhesion and migration. OPN has been noted to play an important role in the progression of interstitial fibrosis by the recruitment and activation of interstitial fibroblasts in the kidney (Yoo et al., 2006). In addition, OPN has been noted to be a promoter of aldosterone-induced inflammation, oxidative stress, and interstitial fibrosis in the kidney (Irita et al., 2011). It is noteworthy that renal mRNA expression of OPN was increased in diabetic conditions and has been shown to induce interstitial fibrosis in the diabetic kidney, suggesting a potential role for OPN in the pathogenesis of diabetic nephropathy (Nagao et al., 2012). p27(Kip1), an inhibitor of cyclin-dependent kinases/cyclin complexes, regulates cell cycle progression. Interestingly, p27(Kip1) is stimulated in mesangial cells under diabetic conditions, and the high glucose-induced stimulation of p27(Kip1) has been noted to induce mesangial cell hypertrophy, suggesting a potential role for p27(Kip1) in the pathogenesis of diabetic nephropathy (Wolf et al.,...
This detrimental role of p27(Kip1) was further confirmed in a study that demonstrated an association between p27(Kip1) and the upregulation of TGF-β1, collagen type IV and laminin expression in diabetic kidneys (Wolf et al., 2005). In addition, the deletion of p27(Kip1) abolished diabetic glomerular hypertrophy through a significant reduction of TGF-β1 expression in the tubulointerstitium (Wolf et al., 2005). Urotensin II (UII) is an 11–amino acid vasoactive peptide that has been identified as the ligand for a novel G protein-coupled receptor. Apart from its basic vasoconstrictive actions, UII also possesses profibrotic properties. Interestingly, UII and its receptors have been noted to be overexpressed in the kidneys of diabetic patients, suggesting a role for UII and its receptor in the pathogenesis of diabetic nephropathy (Langham et al., 2004). The diabetes-induced upregulation of UII and its receptors have been noted to play an important role in TGF-β1-mediated renal fibrosis and dysfunction (Tian et al., 2008). However, in a recently conducted clinical trial, short-term treatment with palosuran, an UII receptor antagonist, did not have a significant effect on urinary albumin excretion. Instead, it was suggested that the potential antifibrotic action of urotensin antagonism might require a longer treatment period (Vogt et al., 2010). Thus, further studies are needed to explore the therapeutic potential of urotensin antagonism in the management of diabetic nephropathy. The mammalian target of the rapamycin (mTOR) signaling cascade, a key component of two multiprotein complexes known as mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), controls cellular growth, survival, and metabolism. Diabetes mellitus has been noted to promote increased p-Akt and mTOR expression (Lloberas et al., 2006), and this over-activation of mTOR signaling has been associated with increased levels of renal mRNA expression of the proliferating cell nuclear antigens TGF-β1, VEGF, and MCP-1. This association resulted in
marked renal structural and functional alterations, including increased albuminuria, glomerular hypertrophy, and glomerular basement membrane thickening. Treatment with rapamycin, an mTOR blocker, significantly reversed these renal structural and functional alterations by inhibiting the reduction of TGF-β1, VEGF, MCP-1 and renal macrophage recruitment (Yang et al., 2007), suggesting that the mTOR signaling cascade promotes the pathogenesis of diabetic nephropathy. The detrimental role of the mTOR signaling cascade and the therapeutic potential of its inhibitor were confirmed by another study that demonstrated a marked amelioration in the pathological changes and renal dysfunction in diabetic mice after treatment with rapamycin (Mori et al., 2009). In addition, the mTOR downstream proteins p-T389-S6K and p-T37/46-4EBP1 were also noted to be over-expressed in the kidneys of diabetic rats, concurrent with the decreased expression of the catalytic subunit of protein phosphatase 2A (PP2Ac) in the diabetic kidney, which has been associated with the occurrence of albuminuria and mesangial expansion. Simultaneous treatment with Sirolimus, an mTOR blocker, and rosiglitazone, a PPAR-γ activator, synergistically reduced renal hypertrophy, albuminuria and renal TGF-β1 by down-regulating the mTORC1 pathway and over-activating PP2Ac in diabetic kidneys (Flaquer et al., 2010). However, further studies are required to explore the therapeutic effectiveness and, most importantly, the safety of mTOR blockage in the clinical management of diabetic nephropathy. Peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors of a nuclear hormone receptor superfamily and consist of three members: PPARα, PPARγ and PPARβ/δ (Desvergne and Wahli, 1999). PPARs play an important role in the general transcriptional control of numerous cellular processes, including lipid metabolism, glucose homeostasis, inflammation, extracellular matrix remodeling. All three PPARs, are expressed in the
kidney (Guan and Breyer, 2001). Reduction in PPARγ and PPARα activity during the diabetic state has been noted to be implicated in pathogenesis of diabetic nephropathy (Zheng et al., 2002; Park et al., 2006). In addition, numerous studies have identified PPARα and PPARγ agonists as novel therapeutic interventions to manage diabetic nephropathy. PPARα agonists have been noted to provide renoprotection by downregulating the renal expression of TGF-β and PAI-1 (Chen et al., 2006), decreasing renal COX-2 expression and reducing nitrosative stress (Chen and Quilley, 2008). In addition, PPARγ agonists have been noted to possess the therapeutic potential to prevent the development of diabetic nephropathy by decreasing the TGF-β-mediated renal upregulation of type1 collagen (Zheng et al., 2002), by downregulating the expression of glomerular fibronectin and inhibiting ROS accumulation in glomeruli of diabetic mice (Zhang et al., 2008a), by decreasing the MMP-2 and MMP-9 activity in glomeruli (Sen et al., 2008), and by suppressing the expression of TGF-β, VEGF, PAI-1, type-IV collagen and ICAM-1 in the kidneys of diabetic rats with nephropathy (Ohga et al., 2007; Ko et al., 2008). Recently, we demonstrated that the simultaneous sub-maximal activation of PPAR-α and PPAR-γ prevents the development of diabetes-induced nephropathy by reducing the altered lipid profile, decreasing the renal oxidative stress, and subsequently improving the function and architecture of the diabetic kidney in rats (Arora et al., 2010).

Numerous efforts have been made to explore the role of the intrarenal dopaminergic system in the pathogenesis of diabetic nephropathy. The dopamine levels were increased in the kidneys of diabetic mice compared to the normoglycemic mice, suggesting a possible role for dopamine in the pathogenesis of diabetic nephropathy (Marco et al., 2008). However, a recent study using COMT(-/-) mice demonstrated that COMT(-/-) mice (which have increased kidney dopamine levels due to the deletion of a major
intrarenal dopamine-metabolizing enzyme) were noted to have decreased albuminuria, renal fibrosis and glomerulopathy, along with inhibited expression of markers of inflammation and oxidative stress. This suggests that decreased renal dopamine production plays a detrimental role in the pathogenesis of diabetic nephropathy (Zhang et al., 2012). In addition, COMT(-/-) mice showed decreased albuminuria and tubulointerstitial injury compared to wild-type mice in response to Ang II infusion (Yang et al., 2012). Further, treatment with losartan has been noted to provide renoprotective effects in diabetic rats through its inhibitory action on monoamine oxidase type A activity, confirming that decreased renal dopamine production may have important consequences in the underlying pathogenesis of diabetic nephropathy (Manni et al., 2012). Therefore, it may be worthwhile to explore the renoprotective effects of intrarenal dopaminergic modulators. Toll-like receptors (TLRs) modulate a crucial role in the pro-inflammatory states of diabetes (Devaraj et al., 2011). The expression of TLR4 has been noted to be elevated in the kidneys of diabetic patients, while the TLR4-mediated pathway has been shown to potentially promote tubulointerstitial inflammation in diabetic nephropathy (Lin et al., 2012). TLR4 KO mice, upon treatment with both streptozotocin and a high fat diet, did not exhibit an increase in albuminuria, mesangial expansion, macrophage infiltration, or the upregulation of pro-inflammatory and extracellular-matrix-associated gene expression in glomeruli in comparison to wild-type mice after treatment with both streptozotocin and a high fat diet (Kuwabara et al., 2012). These results confirm the pathogenic role of TLR4 in the induction and progression of diabetic nephropathy. A summary of potential target sites, along with their downstream signaling pathways for managing diabetic nephropathy, are listed in table 1.
Oxidative Stress

Under normal physiological conditions, approximately 0.1%–5% of oxygen that enters the electron transport chain is reduced to superoxide; a reactive oxygen species (ROS) and the rest is used in metabolic processes and during this state, there is a balance in the generation of oxygen free radicals and antioxidant defense systems used by organisms to deactivate and protect themselves against free radical toxicity. Antioxidant defense system includes ROS degrading molecules (ROS scavengers), such as uric acid, ascorbic acid, and sulfhydryl-containing molecules (e.g. glutathione), and antioxidant enzymes, such as catalase, glutathione peroxidase and superoxide dismutases. Contrarily, main sources of oxidants include redox enzymes such as NADPH oxidase, xanthine oxidase, lipooxygenase and cyclooxygenase. Impairment in the oxidant/antioxidant equilibrium creates a condition known as oxidative stress and results in tissue degeneration, particularly in vascular system.

Diabetes and ROS generation

A large body of evidence indicates that diabetes is a state of increased oxidative stress, and it has been suggested that oxidants are the causative link for the major pathways that have been implicated in vascular complications of diabetes (Evans et al., 2002; King and Loeken, 2004; Pennathur and Heinecke, 2007; Giacco and Brownlee, 2010). Biomarkers for oxidative damage to DNA, lipids, and proteins are also supporting the concept of increased oxidative stress in diabetic complications including diabetic nephropathy. ROS act as intracellular messengers and integral glucose signaling molecules in the diabetic kidney. ROS are formed by incomplete reduction of molecular oxygen. They include superoxide anion (O$_2^-$), hydrogen...
Chapter 2

Review of literature

peroxide ($H_2O_2$), hydroxyl radical (OH), and singlet oxygen ($^1O_2$). Under hyperglycemic condition, glucose in the cytosol is side-tracked to the polyol pathway, where the aldose reductase reduces it to sorbitol by utilizing cofactor NADPH from the pentose phosphate pathway and this excessive consumption of NADPH results in excessive free radical generation (Brownlee et al., 2001). These free radicals further stimulate oxidation of LDL, and ox-LDL, which is not recognized by the LDL receptor and are taken up by scavenger receptors in macrophages leading to foam cell formation and atherosclerotic plaques (Boullier et al., 2001), a consequence of diabetes associated vascular disorders (Figure 5, 6). In addition, under diabetic conditions ROS are produced by the non-enzymatic glycation reaction of proteins, mitochondria, and PKC-dependent activation of NADPH oxidase in mesangial cells, infiltrated inflammatory cells and endothelial cells. Moreover, the persistence of hyperglycemia has been reported to increase the production of ROS through glucose auto-oxidation, abnormal metabolism of prostaglandins, and high polyol pathway flux (Maritim et al., 2003; Forbes et al., 2008). The increased ROS in the kidney, especially the superoxide radicals react with nitric oxide (NO) to form peroxynitrite, which in turn binds to tyrosine and other protein residues, yielding highly cytotoxic compounds such as nitrotyrosine in the renal and other vascular tissues (Pacher et al., 2005). Furthermore, ROS may induce different types of cell injuries, particularly lipid peroxidation and membrane damage. Mechanisms involved in the increased ROS during diabetes mellitus not only increase oxidative damage products of protein, lipid and DNA, but also bring changes in the tissue content and activity of antioxidant defense systems.
RAAS and oxidative stress

Although multiple pathways are involved in the RAAS-dependent renal injury, there is increasing evidence supporting the roles of ROS, which contribute to renal functional aberrations and pro-inflammatory or pro-fibrotic tissue damages. Interestingly, Ang II-induced renal injury is ROS dependent as Nox-4-derived ROS generation was noted to be involved in Ang II-induced protein synthesis in mesangial cells and renal hypertrophy (Gorin et al., 2005; Tabet et al., 2008). In addition to Ang II, animal studies have shown that aldosterone-dependent renal injury is also associated with increased ROS production and increased NADPH oxidase activity. Ang II stimulates upregulation of various NADPH oxidase subunits, including Nox1, p47phox, p67phox, and p22phox, in various cell types by inducing serine phosphorylation of p47phox, resulting in an increased binding of p47phox to p22phox (Dikalova et al., 2005). Whereas, aldosterone increases the ROS formation by increasing expressions of the p47phox and p67phox subunits of NADPH oxidase (Miyata et al., 2005; Sun et al., 2006). Vascular endothelial growth factor (VEGF) has been implicated in the pathogenesis of diabetic nephropathy as VEGF is up regulated early in diabetes mellitus, especially in podocytes and mainly associated with hyperfiltration, proteinuria and glomerular hypertrophy (Cooper et al., 1999; Cha et al., 2004). Ang II mediated ROS generation via activation of NADPH further stimulates the VEGF synthesis (Feliers et al., 2006) suggesting the central role of ROS generation in induction and progression of diabetic nephropathy. In addition, upregulated AT-1 receptor signaling within the kidney has been noted to mediate renal injury by increasing vascular resistance, glomerular capillary pressure, mechanical stretch-induced glomerular injury, ROS production and extracellular
matrix accumulation in the mesangium and tubulointerstitium (Siragy, 2004; Xue and Siragy, 2005). Moreover, Ang II stimulates mitochondrial ROS generation through the opening of mitochondrial K\(_{\text{ATP}}\) channels (mK\(_{\text{ATP}}\)), leading to redoxsensitive activation of MAPK. It is noteworthy that, Ang II enhances the production of ROS through the activation of the NADPH oxidase, which in turn triggers mK\(_{\text{ATP}}\) opening and mitochondrial ROS production ("ROS-induced ROS-release mechanism"), stating the intense relationship between Ang II and oxidative stress (De Giusti et al., 2009). This detrimental role of Ang II or aldosterone induced ROS in the pathogenesis of diabetic nephropathy is confirmed by the fact that inhibition of the RAAS leads to a reduction in markers of oxidative stress.

**PKC and renal oxidative stress**

PKC, serine threonine kinases comprises of a family of at least 15 isoforms, among these isoforms, PKC -\(\alpha\), -\(\beta_1\), -\(\beta_2\), -\(\delta\) and -\(\epsilon\) have been documented to be activated in the glomeruli of diabetic rats as well as in mesangial cells exposed to high glucose (Koya et al., 1997; Babazono et al., 1998; Whiteside and Dlugosz, 2002). PKC has been considered to be a key signaling gist that contributes diversely in the pathogenesis of diabetic nephropathy because PKC activation is involved in regulating a number of vascular functions such as contractility, cell proliferation and extracellular matrix protein synthesis (Rasmussen et al., 1984; Kariya et al., 1987; Huhtala et al., 1990). High glucose-induced ROS in mesangial cells can be effectively blocked by inhibition of PKC, NADPH oxidase, and mitochondrial electron transfer chain complex I, suggesting that PKC, NADPH oxidase, and mitochondrial metabolism all play a role in high glucose-induced ROS generation (Lee et al., 2003). Various antioxidant agents have been noted to inhibit PKC-dependent cellular
responses involved in the induction and progression of diabetic nephropathy (Bursell and King, 1999; Lal et al., 2000; Meier and King, 2000) and several line of evidences directly suggest that PKC-α as well as PKC-β activation is associated with increased NADPH activity and NADPH-dependent superoxide production (Ohshiro, 2006; Thallas-Bonke et al., 2008). Therefore, PKC seems to be a logical candidate for redox modification by oxidants and antioxidants that may in part determine their detrimental and renoprotective activities respectively. Although PKC activation under hyperglycemia is largely related to an increase in de novo synthesis of diacylglycerol and by other mechanisms (Craven and DeRubertis, 1989) but, activation of PKC has also been reported to be regulated sensitively by oxidative stress (Konishi et al., 1997; Ha et al., 2001). ROS could regulate the activation of PKC through redox changes in sulfhydryl groups of cysteine-rich regions of PKC or through activation of phospholipase D, leading to production of diacylglycerol (Studer et al., 1997) and ROS mediated PKC activation in diabetic kidney was further confirmed by the study that demonstrated the treatment with taurine, an antioxidant, effectively inhibiting the membrane translocation of PKC-δ and PKC-ε in streptozotocin-induced diabetic rat glomeruli (Ha et al., 2001). In addition, PKC has been reported to be involved in NADPH oxidase-dependent ROS production in cultured mouse podocytes and mesangial cells exposed to high concentration of glucose (Lee et al., 2006; Xia et al., 2006). It is worthwhile to note that, activation of NADPH oxidase via phosphorylation of PKC-α seems to downstream the AGE–receptor for AGE interaction in diabetic renal disease (Thallas-Bonke et al., 2008) and blockade of NADPH oxidase by apocynin, a NADPH oxidase assembly inhibitor was noted to provide the renoprotective effect as it decreased the renal extracellular matrix accumulation of fibronectin and collagen IV by attenuating the cytosolic superoxide,
PKC activation and increased VEGF (Thallas-Bonke et al., 2008) confirming that PKC activation and ROS generation are unifying in induction and progression of diabetic nephropathy.

**Rho-kinase and oxidative stress**

Rho-kinase, a serine/threonine kinase, is a target protein of the small GTPase Rho and its downstream signaling pathways have emerged as important players in cardiovascular and renal pathophysiology (Shimokawa, 2002; Wakino et al., 2005). The Rho/Rho kinase pathway plays an important role in the structure and function of various kidney cells including tubular epithelial cells, mesangial cells and podocytes (Wakino et al., 2005). Ang II has been noted to cause renal fibrosis by upregulating the expression of Rho-A and activating Rho/Rho kinase pathway (Ruiz-Ortega et al., 2006). Interestingly, Ang II-induced RhoA/Rho-kinase activation was noted to be dependent on the activation of NADPH oxidase (Jin et al., 2006). Activated Rho/Rho-kinase pathway was found to be involved in the up-regulation of TGF-β, CTGF and NADPH oxidase in the diabetic kidney and treatment with fasudil, a Rho-kinase inhibitor markedly attenuated the development of diabetic nephropathy by inhibiting the renal upregulation of TGF-β, CTGF and NADPH oxidase in rats (Gojo et al., 2007). In addition, fasudil treatment has shown no effect on plasma glucose, but it normalized the increased albuminuria and decreased the levels of urinary 8-hydroxyguanosine, a marker of oxidative stress. Further, fasudil prevented diabetes-related increases in mRNA for TGF-β and CTGF as well as the NOX-4 catalytic subunit of NADPH oxidase (Gojo et al., 2007). Taken together, these results not only confirm the detrimental role of Rho/Rho-kinase pathway but also suggest the

**Janus kinase and oxidative stress**

The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway are pleiotropic cascade essential to cytokine and growth hormone receptor signaling. The activation of JAK/STAT has been reported to be an essential pathway for high glucose-induced glomerular mesangial cell growth and activation of JAK/STAT pathway is associated with synthesis of extracellular matrix proteins in glomerular mesangial cells under high glucose condition by increasing the TGF-β and fibronectin synthesis in glomerular mesangial cell (Wang et al., 2002). Concerning activation of this pathway it has been observed that the JAK/STAT pathway responds to intracellular ROS, oxidative stress and it has been suggested that it may be a major contributor in pathogenesis of diabetic nephropathy (Ha and Lee, 2000; Arany et al., 2006). The role of JAK/STAT pathway in oxidative stress mediated renal injury was confirmed as peroxynitrite, a highly reactive oxidant brings the structural and function changes in the kidney of diabetic rats through activation of JAK/STAT pathway (Wang et al., 2009).

**TGF-β and oxidative stress**

Transforming growth factor-β belongs to the TGF-β super-family of multifunctional cytokines and plays an important role in mediating the hypertrophic and fibrotic/sclerotic manifestation diabetic nephropathy. Numerous *in-vitro* as well as *in-vivo* investigations have shown that renal TGF-β production increased during the development of diabetic kidney disease with the increase in the expression of CTGF,
VEGF, collagen I, collagen IV, and fibronectin that results in disassembly and hypertrophy of mesangial cells (Border and Noble, 1998; Hoffman et al., 1998; Goldfarb and Ziyadeh, 2001; Ziyadeh, 2004; Jeong et al., 2004), thereby implicating the pathogenesis of diabetic nephropathy. It is interesting to note that TGF-β1 upregulates the expression of p22phox, p47phox, p67phox, and gp91phox in rat mesangial cells and p22phox mRNA in tubular epithelial cells suggesting that TGF-β1-induced ROS may be NADPH oxidase dependent (Lee et al., 2003). On the other hand, H₂O₂ continuously generated by glucose oxidase upregulates TGF-β1 and fibronectin expression in mesangial cells (Ha and Lee, 2000; Iglesias-De et al., 2001) and fibronectin in tubular epithelial cells and treatment with antioxidants has been noted to inhibit this H₂O₂-induced TGF-β1 and fibronectin upregulation (Studer et al., 1997; Ha and Lee, 2000; Iglesias-De et al., 2001; Ha et al., 1997; Yang et al., 2003), thus providing evidence that ROS plays an important role in high glucose-induced renal injury.

Nitric oxide (NO) contributes to the alterations in glomerular hemodynamics and extracellular matrix accumulation observed in diabetic nephropathy and it was observed that high glucose concentrations directly inhibit NO production in rat mesangial cells (Trachtman et al., 1997). It is worth noting that even in normal glucose media TGF-beta inhibited NO synthesis and TGF-β inhibits iNOS expression at multiple levels i.e it decreases stability of iNOS mRNA, reduces translation of the mRNA, and increases degradation of iNOS protein while other growth factors such as IGF-I, EGF, had no effect on nitrite concentration suggesting the direct involvement of TGF-β in renal NOS generation through modulation of NO (Kitamura and Suto, 1997; Trachtman et al., 1998). In addition, during diabetic state, TGF-β has been noted to be involved in NADPH oxidase-mediated generation of ROS in mesangial
cells exposed to high glucose (Xia et al., 2008). The involvement of TGF-β1 in ROS generation was further confirmed by a study which has demonstrated that TGF-β increases both the activity of NADPH oxidase and expression of Nox-2 and Nox-4, homologs of the NADPH oxidase family (Bondi et al., 2010). Regarding the role of ROS in activation of TGF-β signaling, an object of intense research, it has been observed that NADPH oxidase plays a key role in TGF-β1 activation of kidney myofibroblast and Fn-ED-A expression through Smad3 and ERK signaling pathways (Bondi et al., 2010) telltale the central role of oxidative stress mediators in the pathogenesis of this renal disorder.

**TNF-α and oxidative stress**

Inflammation plays a pivotal role in pathogenesis of diabetic nephropathy. Inflammatory cytokines, mainly IL-1, IL-6, and IL-18, as well as TNF-α, are involved in the development and progression of diabetic nephropathy (Hasegawa et al., 1991; Sekizuka et al., 1994; Navarro et al., 1999; Moriwaki et al., 2003). TNF-α is one of the main proinflammatory cytokines and may be produced intrinsically in renal cells (Nakamura, 1993; Ortiz et al., 1995). Increased level of TNF-α and concomitant and increased oxidative stress in diabetic rat kidney were noted to be associated with increased albumin permeability and urinary albumin excretion, hallmark signature of progression of diabetic nephropathy (Kuhad and Chopra, 2009). In addition to ROS generation, nitrostative stress marker and peroxynitrite levels were also noted to be associated with increased TNF-α levels and increased glomerular lesion in experimental diabetic rats (Xiao et al., 2009) suggesting the role of free radical generation in association with TNF-α in induction and progression of diabetes-associated renal injury. Recently, TNF-α and NF-κB–induced tumor necrosis factor-
α-inducible protein 8 (TNFAIP8), have been noted to be critical components in mesangial cell proliferation during diabetic renal injury and it is interesting to note that NADPH oxidase regulates the renal expression of TNFAIP8 (Zhang et al., 2010). Thus, the potential for TNF-α receptor inhibition in the regulation of NADPH oxidases in diabetic nephropathy is promising and should be further investigated in clinical studies.

**Nuclear factor kappa B and oxidative stress**

Nuclear factor kappa B (NF-kB), comprising of a family of dimeric transcription factors, is involved in the response to oxidative stress and inflammation and its expression has been noted to be increased in kidneys of diabetic animals (Iwamoto et al., 2001), suggesting the possible role of NF-kB activation in pathogenesis of diabetes-induced renal injury. NF-kB can be activated by a wide array of exogenous and endogenous stimuli including hyperglycemia, elevated ROS, Ang II, TNF-α, IL-1β, and other proinflammatory cytokines (Hofmann et al., 1999; Evans et al., 2002). Hyperglycemia-induced NF-kB activation in mesangial cells was noted to be ROS dependent and was confirmed as treatment with various antioxidants such as pyrrolidine dithiocarbamate, N-acetyl-L-cystein, and trolox effectively inhibited hyperglycemis-induced NF-kB activation in mesangial cells (Ha et al., 2002). In addition, AGEs trigger the generation of ROS, and upregulate the NF-kB (Aronson and Rayfield, 2002). During an in-vitro study, performed to investigate involvement of NF-kB in high glucose-induced Na+/glucose cotransporters dysfunction in primary cultured renal proximal tubules cells, it was observed that antioxidants effectively blocked high glucose-induced activation of NF-kB (Han et al., 2003) which further
confirm the central role of oxidative stress in progression of diabetes-induced renal injury.

**MCP-1 and oxidative stress**

MCP-1 is a potent chemokine that plays an important role in the pathogenesis of diabetic nephropathy by inducing renal inflammation and renal extracellular matrix accumulation under diabetic conditions by interacting with its receptor C-C chemokine receptor 2 (Park *et al.*, 2008). It has been noted that high levels of glucose stimulates MCP-1 production by human and mouse mesangial cells through a pathway which involves increased levels of oxidative stress and involvement of oxidative stress in MCP-1. Mediated renal injury was confirmed by the fact that elevated level of oxidative stress increase macrophage recruitment and renal ICAM-1 and MCP-1 expression in diabetic rats (Wu *et al.*, 2006). In addition, during an experiment it has been observed that diabetes mellitus exhibit an increase in oxidative stress and inflammatory cytokines including MCP-1 in mesangial cells of diabetic mice and treatment with antioxidant reduces a MCP-1 level further which confirms the correlation between oxidative stress and MCP-1 in induction and progression of diabetic nephropathy. Moreover, clinical studies also divulge the correlation between oxidative stress and plasma MCP-1 in type 1 diabetic patients with microalbuminuria as vitamin E, an antioxidant treatment reduced plasma MCP-1 and albuminuria in diabetic patients and it is also suggested that the causative role of poor glycemic control in diabetic nephropathy is mediated by increased oxidative stress (Chiarelli *et al.*, 2002; Cipollone *et al.*, 2005). Therefore, these findings point out the pathogenetic role of oxidative stress mediated MCP-1 in the diabetic nephropathy.
Novel antioxidants in diabetic nephropathy

Diabetes has been well studied as a state of increased oxidative stress and treating diabetic nephropathy by targeting ROS generation, responsible for regulating numerous pathways has been identified as an object of intense research, which resulted in the further exploration of putative mechanism of action involved in renoprotective potential of antioxidants against diabetes-induced nephropathy. Vitamin E (20 mg/kg/day for 8 weeks), a natural antioxidant has been noted to ameliorate early diabetic renal hemodynamic abnormality as urinary albumin excretion and creatinine clearance rate significantly decreased via inhibiting PKC (Xu et al., 2001). There are several lines of evidences demonstrating the renoprotective role of antioxidant supplementation in early stages of experimental diabetic nephropathy (Melhem et al., 2001; Melhem et al., 2002; Tian et al., 2012; Zhou et al., 2012). But it is worth noting that delayed treatment with Magnesium lithospermate B, an active component isolated from Salvia miltiorrhizae with antioxidative property, significantly suppresses the progression of albuminuria, glomerular hypertrophy, mesangial expansion, and upregulation of renal TGF-β1, fibronectin, and collagen expression in diabetic rats (Lee et al., 2003a). Treatment with various antioxidants such as catalase, SOD and GSH were noted to inhibit high-glucose-induced VEGF mRNA and protein overexpression in podocytes (Lee et al., 2006). In addition, administration of SOD mimetic was noted to improve the renal and structural abnormality by reducing TNF-α, NF-κB and NOX-1 in type 2 diabetic Zucker rats (Ebenezer et al., 2009). Moreover, a recent study using SOD1-deficient C57BL/6-Akita mice was also noted to be associated with increased renal mRNA expression of TGF-β1 and CTGF, reduced glomerular nitric oxide, and increased renal PGE₂
production (Fujita et al., 2012) suggesting the direct renoprotective effect of exogenous as well as endogenous antioxidant system during diabetic condition. Renoprotective potential of apocynin, a NADPH oxidase assembly inhibitor was noted to be associated with decreased renal extracellular matrix accumulation of fibronectin and collagen IV through attenuation of cytosolic superoxide, PKC activation and increased expression of VEGF (Thallas-Bonke et al., 2008). Moreover, Oil palm (Elaeis guineensis) leaves extract (500mg/kg) possessing antioxidant properties was noted to provide the renoprotection in diabetic rat by decreasing the glomerulosclerosis and tubulointerstitial fibrosis though reduction in plasma TGF-β1 concentration and oxidative markers (Rajavel et al., 2012). Taken together, these studies suggest that antioxidants may provide a new therapeutic advancement for treating diabetic patients with nephropathy. The central role of oxidative stress in the pathogenesis of diabetic nephropathy has been depicted in Figure 7.
PPAR\(\alpha\) Ligands in Diabetic Nephropathy

Activation of PPAR\(\alpha\) induces gene expressions that promote lipid metabolism (Balakumar et al., 2007). PPAR\(\alpha\) is expressed in tissues with high rates of fatty acid metabolism such as liver, heart, kidney, skeletal muscles, brown adipocytes, vascular smooth muscle cells and endothelial cells (Braissant et al., 1996; Ricote et al., 1998; Stael et al., 1998; Marx et al., 1999). PPAR\(\alpha\) plays an important role in the oxidation of fatty acids (Ferre, 2004). Fibrates class of interventions such as bezafibrate, fenofibrate and gemfibrozil are well-known hypolipidemic agents and they are ligands of PPAR\(\alpha\) (Balakumar et al., 2007a; Rose et al., 2007). Numerous studies suggest that hyperlipidemia is an independent risk factor involved in the development of diabetic nephropathy (Ravid et al., 1995; Smulders et al., 1997; Bonnet and Cooper, 2000; Rosario and Sharma, 2006). The elevated levels of lipids are associated in the progression of renal dysfunction (Munter et al., 2000). The increase in circulating lipids induces glomerulosclerosis and tubulointerstitial injury by accelerating the generation of ROS and stimulating the over expression of TGF-\(\beta\) in the glomeruli and tubulointerstitium (Scheuer et al., 2000). It was suggested that circulating lipids are entrapped by extracellular matrix molecules where they undergo the process of lipid peroxidation and generate ROS to induce renal dysfunction (Trevisan et al., 2006). Further evidences revealed that diabetes may mediate renal injury by increasing the expression of SREBP-1, which is responsible for increasing the synthesis of triglycerides and cholesterol in the kidney, that are associated with the upregulation of TGF-\(\beta\), VEGF and extracellular matrix proteins, resulting in glomerulosclerosis and tubulointerstitial fibrosis to induce diabetic nephropathy (Sun et al., 2002; Wang et al., 2005a). It is interesting to note that PPAR\(\alpha\) deficiency appears to aggravate the
severity of diabetic nephropathy through an increase in circulating lipids, inflammation and extracellular matrix formation in diabetic mice (Park et al., 2006). We have recently demonstrated that diabetes mellitus increases the level of circulating lipids, which play a major role in the development of nephropathy in diabetic rats (Arora et al., 2010) and it has suggested that diabetes-induced earlier event of vascular endothelial dysfunction may contribute in the pathogenesis of diabetic nephropathy (Balakumar et al., 2009a,b). Hence, reducing the circulating lipids in diabetic patients may provide a new therapeutic option in managing the diabetic nephropathy. The hypolipidemic agent like PPARα agonists has been suggested as a novel therapeutic intervention to manage diabetic nephropathy. Bezafibrate, an agonist of PPARα has been noted to provide renoprotection by reducing albuminuria and circulating lipid levels in diabetic patients with nephropathy (Nagai et al., 2000). Treatment with fenofibrate, a PPARα agonist was noted to afford renoprotection by reducing the occurrence of albuminuria and glomerular lesions in experimental diabetic mice (Park et al., 2006a). Further, fenofibrate has been noted to provide renoprotection by decreasing the renal COX-2 expression and reducing nitrosative stress in the kidney of diabetic rats with early stages of nephropathy (Chen et al., 2008). The administration of gemfibrozil, a PPARα agonist reduced albuminuria, glomerulosclerosis and tubulointerstitial fibrosis in diabetic mice with nephropathy (Calkin et al., 2006). Recently, we have shown that treatment with fenofibrate prevented the development of diabetic nephropathy by reducing diabetes-induced increase in serum creatinine, blood urea nitrogen and proteinuria and the renoprotective effect of fenofibrate was associated with its actions on reducing the circulating lipids and oxidative stress (Arora et al., 2010). Moreover, we observed that fenofibrate treatment prevented the diabetes-induced pathological changes in the
glomeruli and reduced the mesangial expansion in diabetic rats (Arora et al., 2010). Taken together, these studies suggest the possible therapeutic outcome of hypolipidemic drugs like PPARα agonists in preventing the development and progression of diabetic nephropathy.

**DPP-4 in Diabetic Nephropathy**

DPP-4 is a ubiquitous enzyme and is found in the endothelium of various organs as well as measurable circulating enzymatic activity in plasma in soluble form. Besides GLP-1, many other peptides are also substrates of DPP-4, but the affinity towards GLP-1 is predominant. Thereby, it rapidly cleaves and inactivates GLP-1, which results in a short circulating half-life of the active form of GLP-1. Thereby, therapeutic approaches for enhancing incretin action include degradation-resistant GLP-1 receptor agonists (incretin mimetics), and inhibitors of DPP-4 activity (incretin enhancers). The both approaches have been found to be promising in lowering the glucose level in the experimental as well as clinical studies. It is interesting to note that GLP-1 expression has also been found in the kidney of rats and the rGLP-1 found to inhibit the Na⁺ reabsorption in the proximal tubule and increases glomerular filtration rate in kidneys (Dunphy et al., 1998; Moreno et al., 2002). In addition, GLP-1 has been noted to improve endothelial dysfunction in type 2 diabetic patients with coronary heart disease (Nystrom et al., 2004). Renal TGF-β production is increased during the development of diabetic kidney disease by increasing the expression of CTGF, VEGF, collagen I, collagen IV, and fibronectin that results in disassembly and hypertrophy of mesangial cells (Border and Noble, 1998; Hoffman et al., 1998; Goldfarb and Ziyadeh, 2001; Ziyadeh, 2004; Jeong et al., 2004), thereby implicated in the pathogenesis of diabetic nephropathy. It’s interesting to note that treatment with
exendin-4 (GLP-1 receptor agonists) was found to show the significant reduction in glomerular hypertrophy, mesangial matrix expansion, TGF-β1 expression, and type IV collagen accumulation and associated glomerular lipid accumulation in *db/db* mice (Park *et al.*, 2007) suggesting the therapeutic potential of GLP-1 receptor agonists and inhibitors of DPP-4 in management of diabetic nephropathy. DPP-4 inhibition promotes an attractive therapeutic principle by increasing plasma concentrations of endogenous GLP-1. While GLP-1 receptor agonists are injectable compounds, DPP-4 inhibitors are orally active. DPP-4 inhibitors have been introduced into type 2 diabetes therapy in 2006 with sitagliptin as first substance, followed by vildagliptin and then saxagliptin in 2009 (Gallwitz, 2010). Saxagliptin has the advantage of having a very high selectivity towards DPP-4 and in comparison to sitagliptin and vildagliptin it has a significantly higher potency to inhibit DPP-4 in vitro (Gallwitz, 2010). In accordance to the US-FDA that every investigational antihyperglycemic agent has to demonstrate that it should not have an adverse impact on cardiovascular risk, saxagliptin was the first agent to meet this FDA requirement (Dave, 2011). In addition, ability of saxagliptin to lower HbA1C without increasing hypoglycemic episodes due to its glucose-dependent action is another advantage (Dave, 2011). Taken together, on the basis of safety profile, efficacy and selectivity against DDP-4, saxagliptin may provide a prolific therapeutic strategy in the management of nephropathy in diabetic patients.
Clinical Development of Drugs for Treating Diabetic Nephropathy

An open randomised controlled study using captopril (25 mg/day-100 mg/day) demonstrated that ACE inhibition delayed the development of diabetic nephropathy in normotensive insulin dependent diabetic patients with persistent microalbuminuria (Mathiesen et al., 1991). Further, a randomized, controlled trial comparing captopril with placebo in type-I diabetic patients with macroalbuminuria demonstrated that captopril protects against deterioration in renal function in insulin-dependent diabetic nephropathy (Lewis et al., 1993). Captopril was the first drug approved by FDA in nineties for the treatment of diabetic nephropathy. In a clinical trial named “Microalbuminuria, Cardiovascular, and Renal Outcomes-heart Outcomes prevention Evaluation (MICRO-HOPE)”, ramipril reduced the incidence of albuminuria (Gerstein, 2001). The ‘Candesartan and lisinopril microalbuminuria (CALM)’, a prospective, randomised, parallel group, double blind study, demonstrated that candesartan, at a dose of 16 mg once daily is as effective as lisinopril, at a dose of 20 mg once daily in reducing the blood pressure and microalbuminuria in type 2 diabetes patients with hypertension and the treatment with combination of candesartan and lisinopril was found to be more effective in reducing blood pressure (Mogensen et al., 2000). Irbesartan was shown to be effective in preventing the progression of nephropathy in type 2 diabetic patients (Lewis et al., 2001). In addition, the clinical trial named “Irbesartan Diabetic Nephropathy Trial (IDNT)” demonstrated that irbesartan reduced the urinary protein excretion and exhibited renal protection in patients with diabetic nephropathy (Hunsicker, 2004). “Diabetics Exposed to Telmisartan and Enalapril (DETAIL)” trial demonstrated that telmisartan (80 mg daily) was found to be equally effective as enalapril (20 mg daily)
in providing long-term renoprotection in diabetic patients and suggested that ARBs and ACE inhibitors are clinically equivalent in preventing the development of diabetic nephropathy (Barnett et al., 2004). Moreover, the combination of ACE inhibitor and AT-1 receptor blocker was suggested to be a better therapeutic option in treating diabetic nephropathy (Hunsicker, 2004). The combination of ramipril and candesartan was found to be safe and possess an additive effect in reducing blood pressure, proteinuria and urinary TGF-β excretion in patients with diabetic nephropathy (Song et al., 2005). The “Reduction of Endpoints in NIDDM with the Angiotensin-II Antagonist Losartan (RENAAL)” trial demonstrated that losartan, when combined with conventional antihypertensive agents like diuretics, calcium channel blockers or β-blockers, markedly decreased urinary protein excretion and afforded renal protection in hypertensive patients with nephropathy (Eijkelkamp et al., 2007). Treatment with either lacidipine, a calcium channel blocker, or lisinopril reduced the incidence of albuminuria in moderate hypertensive patients with diabetic nephropathy and lisinopril treatment appeared superior in improving the function of diabetic kidney than lacidipine treatment suggesting that ACE inhibitors are better therapeutic agents in treating diabetic nephropathy than calcium channel blockers (Baba et al., 1990). However, “Japan Multicenter Investigation of antihypertensive treatment for diabetic nephropathy in Diabetics (J-MIND)” trial demonstrated that treatments with either enalapril, an inhibitor of ACE or nifedipine, a calcium channel blocker are equally efficient in reducing the development of nephropathy in patients with hypertensive diabetes (Sabuncu et al., 1998). A recent report revealed that the combinations of trandolapril, an ACE inhibitor and verapamil, a calcium channel blocker; or benazepril, an ACE inhibitor and amlodipine, a calcium channel blocker, markedly reduced albuminuria and urinary albumin/creatinine ratio in hypertensive
patients with diabetic nephropathy (Toto et al., 2008). Addition of spironolactone, an aldosterone receptor blocker, to a regimen of ACE inhibitor or AT-1 receptor blocker in patients with diabetic proteinuria causes reduction in proteinuria and lowers the systolic blood pressure (Saklayen et al., 2008). Treatments with spironolactone, either alone or in combination with ACE inhibitor/AT-1 receptor blocker have been shown to slow down the progression of nephropathy in type II diabetes with a marked antialbuminuric effect (Ustundag et al., 2008). Treatment with aliskiren (150 mg daily for 3 months, followed by an increase in dosage to 300 mg daily for another 3 months), an oral direct renin inhibitor, may have renoprotective effects that are independent of its blood-pressure-lowering effect in patients with hypertension, type 2 diabetes, and nephropathy who are receiving losartan (100 mg daily) (Parving et al., 2008). Taken together, it may be suggested that the management of diabetic nephropathy must include either ACE inhibitors or AT-1 receptor blockers with an additional antihypertensive medications like calcium channel blockers or aldosterone receptor blocker or renin inhibitor.
Table 1  Target Sites with their Associated Downstream Signaling System in the Pathogenesis of Diabetic Nephropathy.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Target Site</th>
<th>Associated Down Stream Signaling</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Renin</td>
<td>↑TNF-α, IL-1β, TGF-β, PAI-1, Fibronectin, Collagen-1, VEGF</td>
<td>(Matavelli et al., 2010; Huang et al., 2006; Zhang et al., 2008; Kang et al., 2011)</td>
</tr>
<tr>
<td>2.</td>
<td>Ang II</td>
<td>↑TGF-β, Laminin, Collagens, PAI-1, RHO A signaling pathway, IL-6, MCP-1, VEGF, NADPH oxidase</td>
<td>(Kagami et al., 1994; Issus Egid, 1996; Kutz et al., 2001; Ortega et al., 2006; Wada et al., 2000; Navarro and Mora-Fernandez, 2000; Kato et al., 1999; Cooper et al., 1999; Cha et al., 2000; Giacchetti et al., 2005)</td>
</tr>
<tr>
<td>3.</td>
<td>Aldosterone</td>
<td>↑TGF-β, VEGF, PAI-1, ROS generation</td>
<td>(Han et al., 2006; Brown et al., 2000; Remuzzi et al., 2008)</td>
</tr>
<tr>
<td>4.</td>
<td>PKC-α</td>
<td>↑VEGF, NADPH oxidase</td>
<td>(Oshiro et al., 2006; Thallas-Bonke et al., 2008)</td>
</tr>
<tr>
<td>PKC-β</td>
<td></td>
<td>↑NOX-2, NOX-4, VEGF, TGF-β, CTGF, NADPH</td>
<td></td>
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<tr>
<td>PKC-δ</td>
<td></td>
<td>↑TGF-β,</td>
<td></td>
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<tr>
<td>PKC-ε</td>
<td></td>
<td>↑VEGF</td>
<td></td>
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<td>5.</td>
<td>TGF-β</td>
<td>↑PAI-1, Fibronectin, MMP-2, CTGF, Smad2/3, MAPK Signaling, NADPH oxidase ↓PA, Smad7 Signaling</td>
<td>(Schiffer et al., 2002; Isono et al., 2002; Wang et al., 2005; Lan and Chung, 2011; Fujimoto et al., 2003; Li et al., 2004; Tojo et al., 2007; Atfi et al., 1997; Choi, 2000; Chin et al., 2001)</td>
</tr>
<tr>
<td>S. No</td>
<td>Target Site</td>
<td>Associated Down Stream Signaling</td>
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<td>6.</td>
<td>NADPH</td>
<td>↑Fibronectin, Collagen-4, Akt/PKc, ERK1/2, TNF-α, IL-8</td>
<td>(Asaba et al., 1998; Zhang et al., 2010; Gorin et al., 2005)</td>
</tr>
<tr>
<td>7.</td>
<td>TNF-α</td>
<td>↑TGF-β, MCP-1, CTGF, Upregulating of Nephrin ↓PA</td>
<td>(Huwiler et al., 2003; Vassalli, 1992; Feldmann et al., 1988; Colucci et al., 1993; Lin et al., 2005)</td>
</tr>
<tr>
<td>8.</td>
<td>JAK-STAT</td>
<td>↑MCP-1, NF-κβ, ICAM-1, TGF-β</td>
<td>(Lu et al., 2009)</td>
</tr>
<tr>
<td>9.</td>
<td>SOD</td>
<td>↑TGF-β, CTGF</td>
<td>(Fujita et al., 2012)</td>
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<tr>
<td>10.</td>
<td>p27 (Kip1)</td>
<td>↑TGF-β, Collagen Type-4</td>
<td>(Wolf et al., 2005)</td>
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<td>11.</td>
<td>UrotensinII receptor</td>
<td>↑TGF-β</td>
<td>(Tian et al., 2008)</td>
</tr>
<tr>
<td>12.</td>
<td>PPARs</td>
<td>↑TGF-β, PAI-1, ROS, COX-2, VEGF, ICAM-1</td>
<td>(Chen et al., 2006; Chen and Quilley, 2008; Ohga et al., 2007; Ko et al., 2008)</td>
</tr>
<tr>
<td>13.</td>
<td>mTOR signaling cascade</td>
<td>↑TGF-β, MCP-1, VEGF</td>
<td>(Yang et al., 2007)</td>
</tr>
</tbody>
</table>
Figure 1. The Molecular Mechanism Involved in Induction and Progression of Diabetic Nephropathy.

TNF-α indicates tumor necrosis factor-α; Ang II indicates Angiotensin II; TGF-β indicates transforming growth factor-β; NADPH Oxidase indicates nicotinamide adenine dinucleotide phosphate-oxidase; PKC indicates protein kinase C; MCP-1 indicates monocyte chemoattractant protein-1; JAK-STAT signaling indicates janus kinases-signal transducer and activator of transcription signaling; IL-1β indicates interleukin-1β; PAI-1 indicates plasminogen activator inhibitor-1; MCP-1 indicates monocyte chemoattractant protein-1; VEGF indicates vascular endothelial growth factor; ICAM-1 indicates intercellular adhesion molecule-1; NF-κB indicates nuclear factor kappa B; PA indicates plasminogen activator; MMP-2 indicates matrix metalloproteinase-2; ROS indicates reactive oxygen species.
Figure 2. The Signaling Mechanism involved in RAAS-mediated Induction and Progression of Diabetic Nephropathy.

TNF-α indicates tumor necrosis factor-α; IL-1β indicates interleukin-1β; NADPH Oxidase indicates nicotinamide adenine dinucleotide phosphate-oxidase; TGF-β indicates transforming growth factor-β; VEGF indicates vascular endothelial growth factor; PAI-1 indicates plasminogen activator inhibitor-1
Figure 3. The Role of Protein Kinase C isoforms in the Pathogenesis of Diabetic Nephropathy.

PKC indicates protein kinase C; VEGF indicates vascular endothelial growth factor; NADPH Oxidase indicates nicotinamide adenine dinucleotide phosphate-oxidase; TGF-β indicates transforming growth factor-β.
Figure 4. Central Role of TGF-β in Induction and Progression of Diabetic Nephropathy.

JAK-2 indicates janus kinase-2; ERK indicates extracellular regulating kinase; p38 MAPK indicates mitogen-activated protein kinase; VEGF indicates vascular endothelial growth factor; CTGF indicates connective tissue growth factor; PAI-1 indicates plasminogen activator inhibitor-1; MMP-2 indicates matrix metalloproteinase-2; IL-8 indicates interleukin-8; GFR indicates glomerular filtration rate.
Figure 5. The Mechanism Involved in ROS Generation during Hyperglycemia.
Figure 6. The Signaling Mechanism involved in ROS-mediated Diabetic Complication.
Figure 7. Central Role of Oxidative Stress in Pathogenesis of Diabetic Nephropathy.

Ang II indicates Angiotensin-II; PKC indicates protein kinase C; TGF-β indicates transforming growth factor-β; NADPH Oxidase indicates nicotinamide adenine dinucleotide phosphate-oxidase; JAK/STAT indicates janus kinases-signal transducer and activator of transcription; TNFAIP8 indicates TNF-α, NF-κB–induced tumor necrosis factor-α-inducible protein 8; NF-κB indicates nuclear factor kappa B; MCP-1 indicates monocyte chemoattractant protein-1.