Wnt signaling and podocyte dysfunction in diabetic nephropathy

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ABSTRACT

Nephropathy is a major microvascular complication of diabetes mellitus and often leads to terminal renal failure in addition to contributing significantly to cardiovascular morbidity and mortality. Despite continuous advances, the pathogenesis of diabetic nephropathy remains poorly understood. Recent studies have underscored the significance of structural and functional changes in podocytes in the development and progression of diabetic nephropathy. The role of podocytes in health and diabetic nephropathy and abnormalities including podocyte hypertrophy, effacement, and apoptosis, and a detailed discussion on the role played by the Wnt-β-catenin signaling pathway in podocyte injury and dysfunction are the focus of this review. In addition, the role played by Wnt signaling in mediating the effects of known therapeutic strategies for diabetic nephropathy is also discussed.

INTRODUCTION

Diabetic nephropathy (DN) is a major complication of diabetes resulting from long-standing uncontrolled diabetes, often leading to end-stage renal failure. It is characterized clinically by progressive proteinuria, hypertension, and renal failure leading to edema, and uremic symptoms. Histologically, the main features of DN include increased thickness of the basement membrane, mesangial expansion due to accumulation of extracellular matrix (ECM) along with hypercellularity, which ultimately leads to interstitial fibrosis and glomerular sclerosis. Among the different mediators responsible for progression of DN, oxidative stress, hyperglycemia, activation of growth factors such as transorming growth factor-beta (TGF-β), glomerular hypertension, and generation of advanced glycation end products and activation of several signaling pathways are considered to play a vital role in the pathogenesis of renal injury.[1] Different pathways such as TGF-β/Smad signaling pathway, PI3K/Akt, p38 mitogen-activated protein kinas (MAPK), and janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling have been well established in the pathogenesis of DN. A number of recent studies have implicated the critical role of Wnt/β-catenin signaling, a multifunctional pathway, in diabetic renal injury particularly to mesangial cell (MC), podocyte, and tubular cell damage. Wnt signaling has also been involved in renal interstitial fibrosis and glomerular sclerosis, the pathological hallmarks of DN.[2 3] Among the renal cells involved in kidney function and disease, podocytes are of prime importance particularly in proteinuric diseases including DN.

PODOCYTES IN NORMAL KIDNEY

Podocytes are terminally differentiated specialized pericyte-like cells that encase the exterior basement membrane of glomerular capillary.[4] They possess a unique complex cellular morphology which can be divided into cell body, major processes and foot processes. The cell body consists of nucleus, mitochondria, Golgi apparatus, and endoplasmic reticulum, whereas the major processes ascend from cell body which comprised microtubules and vimentin intermediate filaments. The major processes after additional branching split into foot processes which contain three distinct membrane segments: the basal side, the apical side, and slit diaphragm. The basal side contains several types of integrins and dystroglycans (DGs) that helps the podocyte connect to the glomerular basement membrane (GBM)(figure 1). The apical side of the foot process consists of proteins including podocalyxin, sialoglycoprotein, and podoplanin that contribute to charge selectivity as these proteins are negatively charged.[5] The slit diaphragm is a specialized intercellular junction formed by foot processes which interdigitate with each other as well as with neighboring podocytes. These structures primarily function to form a charge and size-selective barrier for proteins permitting permeability to molecules smaller than albumin and also contribute to charge selectivity when the proteins are phosphorylated. Recent studies have highlighted the importance of podocytes in normal kidney function by demonstrating that the function of slit diaphragm as a size barrier is critical for successful retention of albumin and other proteins. The proteins in the slit diaphragm also interact with actin cytoskeleton which affects podocyte motility as well as cellular signaling. It has been established that mutations or deletions in slit diaphragm proteins or slit diaphragm-associated proteins lead to foot process effacement and podocyte dysfunction resulting in defective glomerular filtration along with the onset of proteinuria.[6 7] Thus,

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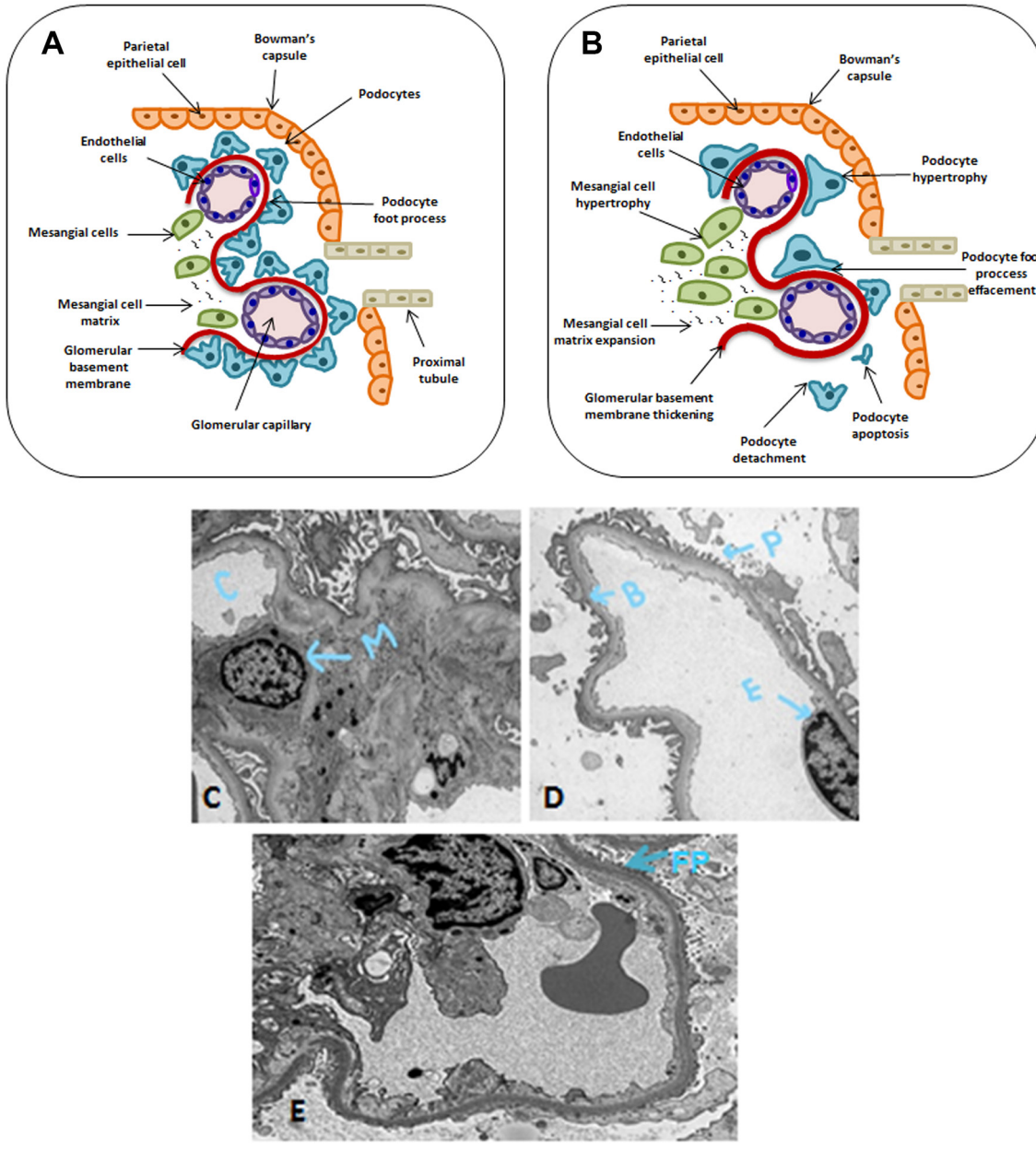

Figure 1 Illustration of structural elements of glomerulus. [A] Normal glomerulus; [B] glomerulus in diabetic nephropathy (DN). Photomicrographs of structural elements of glomerulus [C] illustrating mesangial cells (M) and glomerular capillary (C); [D] showing glomerular basement membrane B, podocytes (P) and endothelial (E)cells ; [E]showing podocyte foot process effacement in DN.

podocytes represent an important cell type in the glomerulus that play a major role in physiological glomerular filtration function and their derangements in pathological states lead to proteinuria.

PODOCYTE DYSFUNCTION IN DN

Recent studies have indicated that podocyte injury or podocytopathy, which includes changes in either the structure, function, or their number in the glomerulus, plays a critical role in the pathogenesis of DN. Specifically podocytes have been found to contribute to glomerular hypertrophy, glomerulosclerosis, and foot process effacement in DN (figure 2). Podocytes are detected in the urine samples of patients with diabetes, and the quantity of podocyturia has been correlated with the severity of the disease.[7]

Podocyte hypertrophy

With long-term uncontrolled diabetes, kidney size is increased primarily due to glomerular and tubular hypertrophy.[8] As part of glomerular hypertrophy, podocytes undergo hypertrophy, by increasing cell size. A recent study suggested that mechanical stretch in wild-type and single p27/podocytes in vitro reduced progression of cell-cycle and induced hypertrophy.[9] While podocyte hypertrophy is well known to be induced by high glucose, there is evidence suggesting that the effect can be blocked by angiotensin

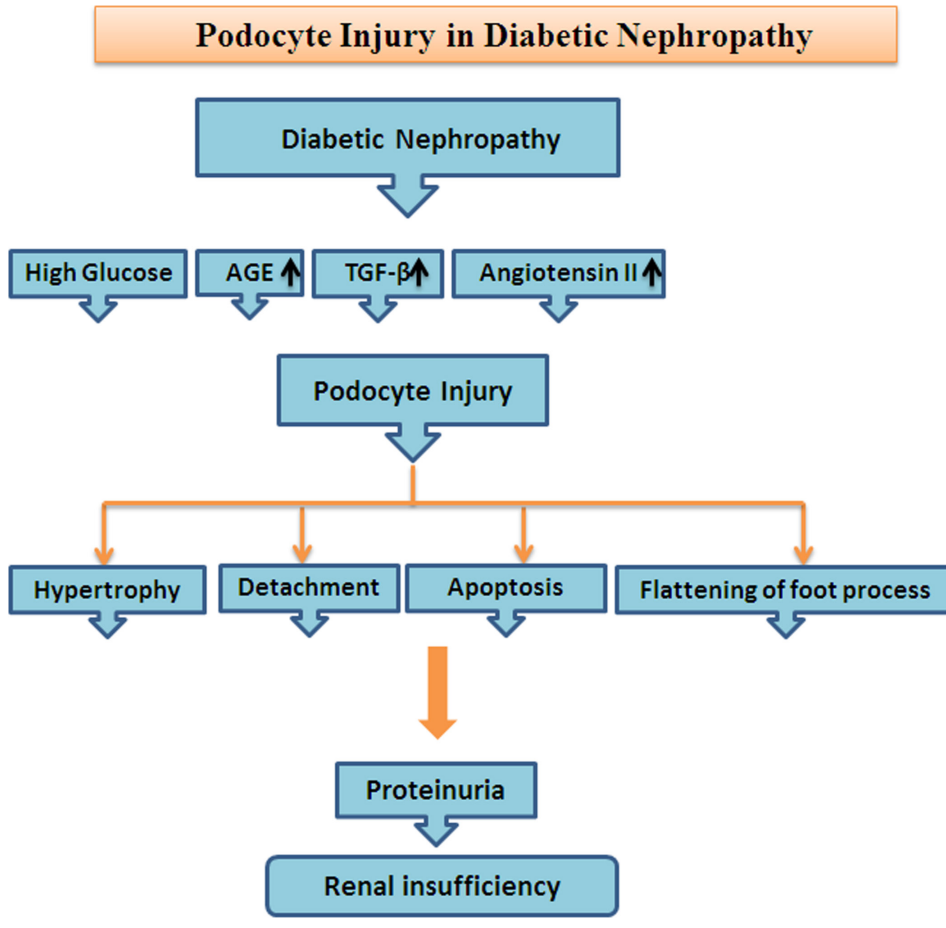

Figure 2 Podocyte injury and loss in diabetic nephropathy.

type I receptor blockers, indicating a role for angiotensin II in glucose-induced podocyte hypertrophy.[10] More recent studies indicated that angiotensin II levels were elevated in podocytes exposed to high glucose along with increased expression of angiotensinogen and angiotensin II type I receptors. These observations implied that high glucose activated local renin–angiotensin system in podocytes.[11]

Podocytopenia

Podocyte loss or podocytopenia refers to the reduced number of podocytes per glomerulus that contributes to podocyte dysfunction in DN.[12] Reduction in podocyte number is primarily a consequence of podocyte loss due to detachment and apoptosis and since podocytes do not proliferate even in normal conditions podocyte number progressively decreases in DN. Previous studies have shown that podocyte loss initiates glomerulosclerosis, followed by heavy protein leakage through the GBM. The loss of podocytes in turn leads to subendothelial hyalinosis of the affected capillary along with formation of tuft adherence to Bowman's capsule.[8 13]

Podocyte detachment

Podocyte proteins such as α3β1 integrin and DGs play a vital role in attachment of podocytes to GBM. Defects in α3β1 integrin and/or DGs therefore could result in podocyte detachment and loss. Reduced α3β1 integrin expression in podocytes has been demonstrated at an early stage of diabetes in humans and rats.[14] α3β1 integrin usually binds to the podocyte actin system through intracellular proteins, one of which is known as talin-1. Deletion of podocyte talin-1 resulted in reduced activation of β1 integrin, and podocyte cell adhesion along with alteration in actin cytoskeleton of podocytes.[15 16] Moreover, deletion of β1 integrin also leads to progressive podocyte loss. Thus, disruption in podocyte integrin interaction leads to podocyte detachment.[17] Integrin β1 subunit and integrin-linked kinase have also been reported to modulate cell adhesion, migration, and phenotypic change, implying this axis might play a role in the pathogenesis of DN.[14 18] However, Sawada *et al*[19] reported that upregulation of α3β1 integrin expression occurred only in podocytes at an early stage whereas the expression decreased at an advanced stage. The precise role of DGs in DN is yet to be fully determined.

Podocyte apoptosis

Podocyte apoptosis has been shown to be induced by hyperglycemia which also leads to glomerular hyperfiltration at the early stages of diabetes. However, as podocyte depletion also leads to nodular and diffuse glomerulosclerosis at the later stages of DN, podocyte apoptosis may have a crucial role in DN both at the early and late stages.[20] Researchers have shown that TGF-β induces apoptosis in cultured podocytes by stimulating MAPK p38 signaling and classic effector caspase-3 pathway.[21] Moreover, TGF-β1 has been

reported to induce podocyte apoptosis through transcriptional stimulation of NADPH oxidase 4 (Nox4) via activation of Smad pathway.[22] Podo cyte apoptosis has also been shown to be induced by angiotensin II via a C-terminal Src kinase-dependent pathway.[23] Liu *et al*[24] have shown that high glucose level induced podocyte apoptosis by stimulating transient receptor potential canonical 6 (TRPC6) channel-mediated elevation of intracellular Ca(2+) in the presence of elevated reactive oxygen species (ROS) levels. Podocyte apoptosis may be reduced by blocking the toll-like receptor (TLR) pathway. An immunomodulatory agent GIT27, for example, prevents downregulation of 3-phosphoinositide-dependent kinase-1[7] (a cell survival factor) and cyclin-dependent kinase 2 [25] thereby ameliorating progression of DN.

Foot process effacement

Podocyte foot process effacement entails retraction, widening, and shortening of the foot processes of each podocyte[8] and is a consistent characteristic of proteinuric glomerular diseases including DN. This phenomenon is featured by the loss of the usual interdigitating pattern of foot processes between the neighboring podocytes leading to short irregularly shaped cell projections. Slit diaphragms are lost or displaced from their basal position and appear to be replaced by occludens-type junctions among the malformed, broadened foot processes thus causing leakage of macromolecules such as albumin through the glomerular filtration barrier.[26] Mutations in nephrin, podocin, and CD2-associated protein which interacts with T lymphocyte and natural killer-specific membrane protein CD2 have been shown to lead to foot process effacement indicating that slit diaphragm proteins are involved in the pathogenesis of foot process effacement.[27]

In summary, several structural changes in the podocytes including hypertrophy, foot process effacement, detachment, and loss of these cells in urine leading to podocytopenia and apoptosis constitute the major changes in the glomeruli of DN. These changes are directly implicated in the development of proteinuria and glomerulosclerosis.

WNT/B-CATENIN SIGNALING

The Wnt signaling pathways are a group of highly conserved signal transduction pathways that modulate several developmental processes in different organs including the kidney. These pathways regulate cell-to-cell interactions particularly during embryonic and fetal development that include cell polarization and basement membrane synthesis and thereby mediate multiple physiological and pathological processes, such as inflammation, angiogenesis, and fibrosis.[28] The term Wnt has been derived from the Drosophila segment polarity gene *wingless* and the name of the vertebrate homolog, *integrated* or *int-1*.[29 30] Even though the pathway has been discovered nearly 30 years ago, it still attracts immense scientific interest and investigation. Wnt signaling is generally inclusive of two major transduction cascades: the Wnt/β-catenin-dependent or canonical pathway and the β-catenin-independent or non-canonical pathways, which in turn includes the planar cell polarity and the Wnt/calcium pathway. The Wnt/β-catenin pathway is integral to the development of several organs and is characterized among the other Wnt pathways in multiple diseases.

Active and inactive state of Wnt/β-catenin signaling

The canonical or β-catenin Wnt pathway regulates gene transcription involved in cell survival, proliferation, and differentiation neurogenesis and inflammation. The functioning of this critical Wnt pathway depends on the regulation of the transcriptional co-activator β-catenin that controls the above-mentioned gene expression programs.[28] The activation of Wnt pathway involves the Wnt family of proteins which are secreted lipid-modified glycoproteins, highly conserved across different species, from metazoans to humans. The Wnt ligands are secreted from neurons and immune cells in the central nervous system acting as signaling proteins in a concentration-dependent manner to activate the intracellular Wnt signaling.[29] In mammalian species, there are 19 different Wnt proteins that have been identified to induce Wnt/β-catenin signaling. Among the different members, Wnt1, Wnt2, Wnt3, Wnt3a, Wnt7a, Wnt8b, and Wnt10b are responsible for activation of β-catenin-dependent canonical pathway, while Wnt4, Wnt5a, and Wnt11 are ligands for the non-canonical pathway. In absence of binding of Wnt ligands to its receptor complex, the Wnt/β-catenin pathway is in an inactive state (figure 3).[1 29 30] Extracellular Wnt inhibitors such as secreted Frizzled-related protein family, Wnt inhibitory factor−1, and Cerberus primarily bind to the Wnt proteins thereby preventing its binding to its receptors. As a consequence, β-catenin is phosphorylated at N-terminal residue by the destruction complex which contains proteins such as axin, adenomatosis polyposis coli, casein kinase-1 (CK-1), and glycogen synthase kinase (GSK-3β). Phosphorylated β-catenin is recognized by F-box/WD repeat-containing protein (β-TrCP), a member of E3 ubiquitin ligase complex which mediates proteosomal degradation of β-catenin. In the nucleus, transcriptional co-repressor proteins like Groucho remains bound to transcription factors T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) which inhibits transcription of Wnt target genes.

When Wnt proteins bind to its transmembrane receptors Frizzled (Fzd) receptor family of proteins, and co-receptors, low-density lipoprotein-related protein 5 and 6 (LRP-5/6), the Wnt signaling pathway is activated.[1] As a consequence of binding of Wnt proteins to its receptor, Wnt signal is transduced to the cytoplasmic phosphoprotein, Disheveled (Dsh/Dvl). Subsequently, the Wnt signal bifurcates into the canonical or β-catenin-dependent pathway or non-canonical or β-catenin-independent pathway. In β-catenin-dependent pathway, once Dvl is phosphorylated, it inhibits the 'destruction complex'. This leads to prevention phosphorylation by the destruction complex and ubiquitin-mediated proteolytic degradation of β-catenin. Unphosphorylated β-catenin thus stabilizes and accumulates in the cytoplasm and finally translocates into the nucleus wherein it binds to TCF/LEF displacing Groucho and stimulates transcription of Wnt downstream target genes.

WNT/B-CATENIN SIGNALING IN NORMAL KIDNEY

The normal mammalian kidney development is initiated from the ureteric bud and mesenchymal

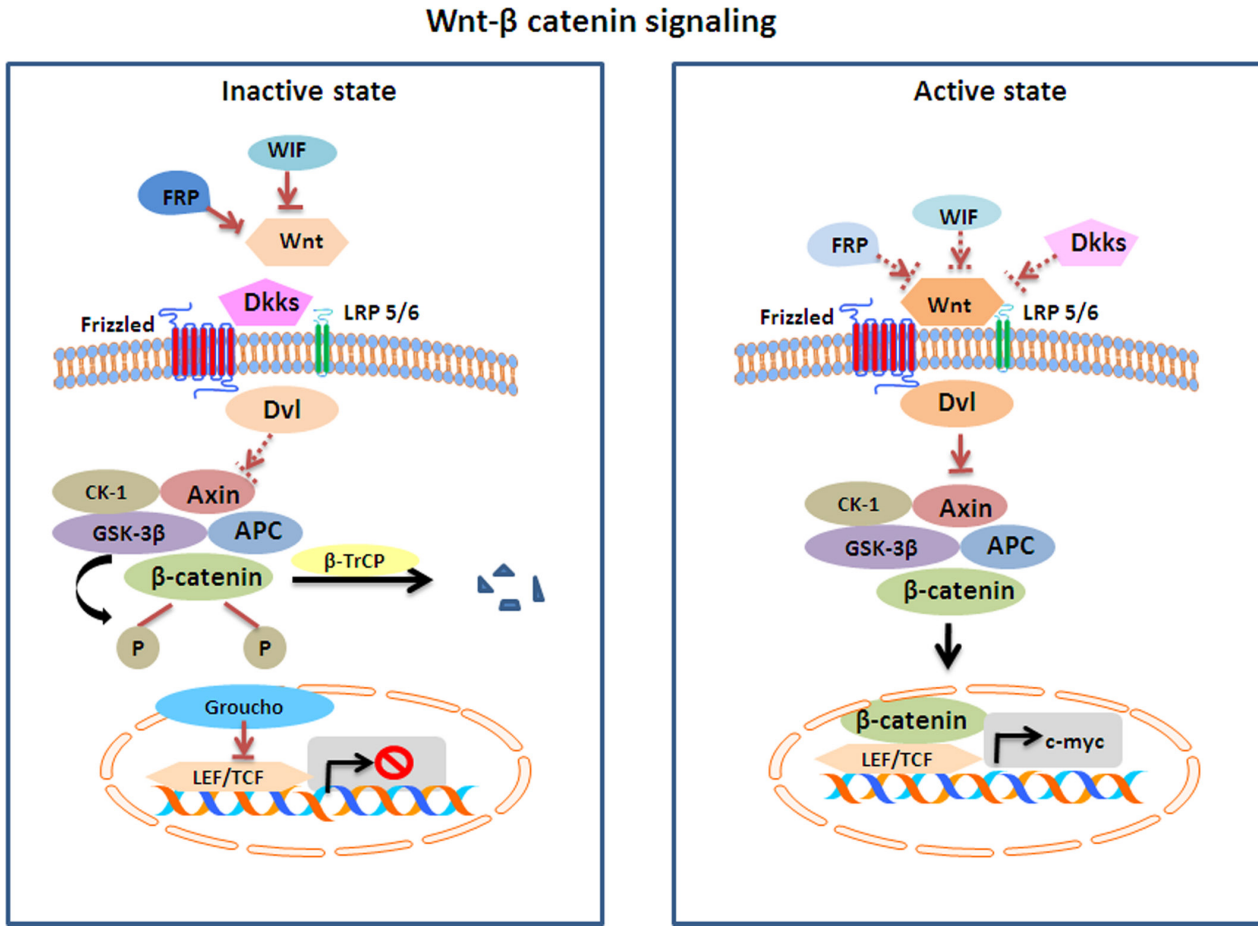

Figure 3 Wnt/β-catenin signaling showing both inactive and active states. APC, adenomatosis polyposis coli; CK-1, casein kinase-1; FRP, Frizzled-related protein; GSK, glycogen synthase kinase; LEF, lymphoid enhancer-binding factor; LRP, lipoprotein-related protein; TCF, T-cell factor; WIF, Wnt inhibitory factor.

epithelial transformation for formation of nephrons. The Wnt pathway, which encodes secreted growth and differentiation factors, has been found to play a critical role in the developmental processes. Among the Wnt family members, Wnt-2b, 4, 5b, 6, 7b, 9b, and 11 have been shown to be expressed during kidney ontogeny[31] while Wnt-6, 7b, 9b, and 11 are expressed in the earlier stages of nephrogenesis in the branching ureteric bud. Studies have indicated that Wnt9b and Wnt4 are absolutely essential for nephrogenesis as they stimulate mesenchymal to epithelial differentiation that subsequently generate nephrons.[32] Wnt9b is necessary for renal vesicle induction, including the activation of Wnt4 while Wnt4 seems to play as an autoinducer of the mesenchymal cells, required for propagation and completion of the transition to epithelial renal vesicles.[33] Regulation of Wnt signaling by Dickkopf-related protein 1 (Dkk1) and axin has also been reported during nephrogenesis. Furthermore, Wnt pathway has been found to be continuously active in renal papillary cells where low oxygen tension causes cell stress with high rate of cell turnover.[4]

In summary, Wnt/β-catenin signaling system is a complex signal transduction pathways comprising canonical and non-canonical pathways. The canonical β-catenin pathways play a major role in nephrogenesis by participating in cell growth, differentiation and proliferation, and in the development of nephrons.

WNT/B-CATENIN SIGNALING IN DN

Evidence is emerging regarding the activation of Wnt/β-catenin pathway in DN showing that levels of β-catenin and Wnt proteins are increased in kidney tissues of animals in type 1 and type 2 diabetic models. The expression of target genes of Wnt signaling pathway has also been studied in kidney diseases including DN. Cell cycle kinase activator cyclin D1 and the transcription factor c-myc are the best-characterized direct downstream genes of Wnt signaling that have been related to kidney injury.[34 35] The activation of direct Wnt target genes generally occurs via β-catenin and TCF with the presence of TCF/LEF-binding site in the promoter region of the target gene. LEF1 mRNA expression was found to increase significantly in glomeruli of patients with diabetic kidney disease.[36] C-myc and cyclin D1 generally play key roles in cell proliferation and cell cycle progression. A study conducted by Rooney *et al* showed that TCF and c-myc gene expression significantly increased in kidney biopsies of patients with diabetes.[2] More downstream direct Wnt targets that have been associated with renal fibrosis

include Snail1, MMP-7 and plasminogen activator inhibitor-1 (PAI-1), and fibronectin.[35] Snail1, the main transcription factor involved in epithelial–mesenchymal transition (EMT), has been found to be upregulated by β-catenin in DN.[1] MMP-7, a member of matrix metalloproteinase (MMP) family that degrades ECM proteins, is generally secreted at low levels in normal tissues. In patients with DN, increased expression of MMP-7 has been observed in kidney biopsy samples along with increased β-catenin expression.[37] PAI-1, a secreted acute phase glycoprotein, inhibits both tissue-type and urokinase-type plasminogen activators. This target gene has a TCF/LEF-binding site in its promoter region and is found to be highly expressed in DN. It has been shown that disruption of the TCF/LEF-binding site or β-catenin signaling inhibited induction of PAI-1.[35 38 39] Fibronectin, the fibrotic matrix protein that binds to integrin and ECM proteins, has also been shown to accumulate in DN by binding of β-catenin to TCF/LEF which initiates this target gene transcription.[40 41] Additional target genes of Wnt/β-catenin activation that result in podocyte injury in diabetes include TRPC6 (transient receptor potential cation channel, subclass C, member 6) and angiotensin II type 1 receptor. TRPC6, a calcium channel, which when mutated or exposed chronically to high glucose result in proteinuria, through a mechanism that involves Wnt/β-catenin activation. Finally, Wilms tumor 1, a transcription factor expressed on podocytes, is downregulated by Wnt/β-catenin activation though ubiquitin pathway and lead to podocyte injury, possibly even in DN. Thus, several downstream genes targeted by Wnt/β-catenin activation continue to be reported and this field represents an area of ongoing research.

Insulin has been found to ameliorate activation of Wnt signaling by reducing blood glucose levels. Furthermore, high glucose levels could activate Wnt signaling in in vitro culture of human renal proximal tubular epithelial cells. Anti-LRP6 antibody attenuated renal inflammation, reduced proteinuria and ameliorated fibrosis. Among the renal cell types which participate in DN and involves Wnt/β-catenin signaling, MCs, tubular cells, and podocytes are particularly important.

The mesangium, which provides structural support to the glomerular capillaries, is composed of MCs and ECM and has major functional significance in health and disease. Progressive mesangial matrix expansion and MC apoptosis are prominent features of DN.[42] Wnt/β-catenin signaling pathway plays a significant role in MC apoptosis in DN. Activation of GSK-3β and degradation of β-catenin along with inhibition of Wnt4 or Wnt5a by hyperglycemia has been found to lead to MC apoptosis. This has been evident from an experiment with simvastatin, a cholesterol-lowering agent, which prevented high glucose-induced activation of Wnt/β-catenin signaling and reduced MC apoptosis.[43] In the study, simvastatin restored Wnt4 and Wnt5a mRNA expression in MCs, which was upregulated with exposure to high glucose. The statin also suppressed high glucose-induced GSK-3b activation followed by restoration of nuclear β-catenin levels. Moreover, TGF-β1 mediated high glucose-induced MC phenotypic transition, a prominent feature in the progression of DN. Mu *et al*, showed that among the microRNAs responsible for EMT in renal cells, miR-215 is involved in TGF-β1-induced mouse MC phenotypic transition. It results in β-catenin activation due to inhibition of CTNNBIP1 (β-catenin interacting protein 1) which in turn blocks Wnt/β-catenin signaling.[1 40]

Apart from MCs, the renal tubular cells are also involved in DN. EMT formation in tubular epithelial cells leads to tubule-interstitial fibrosis. Wnt/β-catenin has been shown to take part in EMT formation in the renal tubular epithelial cell under high glucose induction. Moreover, Wnt upregulation in proximal tubular epithelial cells has been shown to play a role in tubular fibrosis. Activation of Wnt4 in the interstitial area under high glucose condition subsequently leads to tubule-interstitial fibrosis.[44] Although most studies focused on the signaling mechanisms directed toward mesangial expansion and tubular fibrosis in DN, signaling pathways mediating podocyte damage have recently emerged as the early primary event in DN which ultimately leads to end-stage renal failure.

WNT/B-CATENIN SIGNALING CONTRIBUTES TO PODOCYTE DYSFUNCTION IN DN

Wnt proteins and pathways have been found to play a vital role in regulation of glomerular podocyte motility, adhesion, as well as apoptosis. Moreover, β-catenin plays a critical role in regulating podocyte differentiation from renal vesicles and podocyte differentiation markers like nephrin (figure 4). Wnt β-catenin signaling has been shown to be upregulated in podocytes of DN in humans and streptozotocin (STZ)-induced diabetic mice.[45] Although it has been reported that β-catenin expression is necessary for normal GBM functions, studies have proposed that upregulation of Wnt/β-catenin signaling leads to podocyte dysfunction in DN. Expression of Wnt proteins, such as Wnt1,Wnt2B, Wnt4,Wnt6, and Wnt16, has been found to be upregulated in experimental DN. Furthermore, increased expression of Wnt1 and β-catenin has also been observed in the podocytes of DN animal models. While activation of Wnt/-catenin led to podocyte dysfunction, inhibition of Wnt signaling by Dkk1 restored podocyte function and decreased albuminuria, implicating a significant role for Wnt/β-catenin in podocyte damage in DN. It has also been shown that β-catenin deletion in podocytes and overexpression of Dkk1 led to increased severity of STZ-induced DN suggesting that both hypoactivation and hyperactivation of Wnt signaling promoted renal injury in DN.[41]

Evidence from a previously published study indicated that there is a correlation between activation of Wnt/β-catenin pathway and canonical TRPC6 in mediating podocyte injury in DN.[46] TRPC6 is a key protein responsible for calcium flux in the podocytes which on activation results in excessive calcium influx in podocytes leading to foot process effacement, podocyte apoptosis, and subsequent glomerular damage.[47] It has been shown that podocyte apoptosis and decrease in viability of differentiated mouse podocytes in the presence of high glucose caused increased expression of TRPC6 and activation of Wnt/β-catenin pathway. However, blockade of the Wnt signaling pathway by Dkk1 resulted in reduced expression of TRPC6 and thereby amelioration of podocyte apoptosis and viability.[47] Angiotensin II (Ang II) also has also been shown to induce Wnt1 expression and β-catenin nuclear translocation in mouse podocyte culture. Blockade of Wnt signaling by Dkk-1 or β-catenin

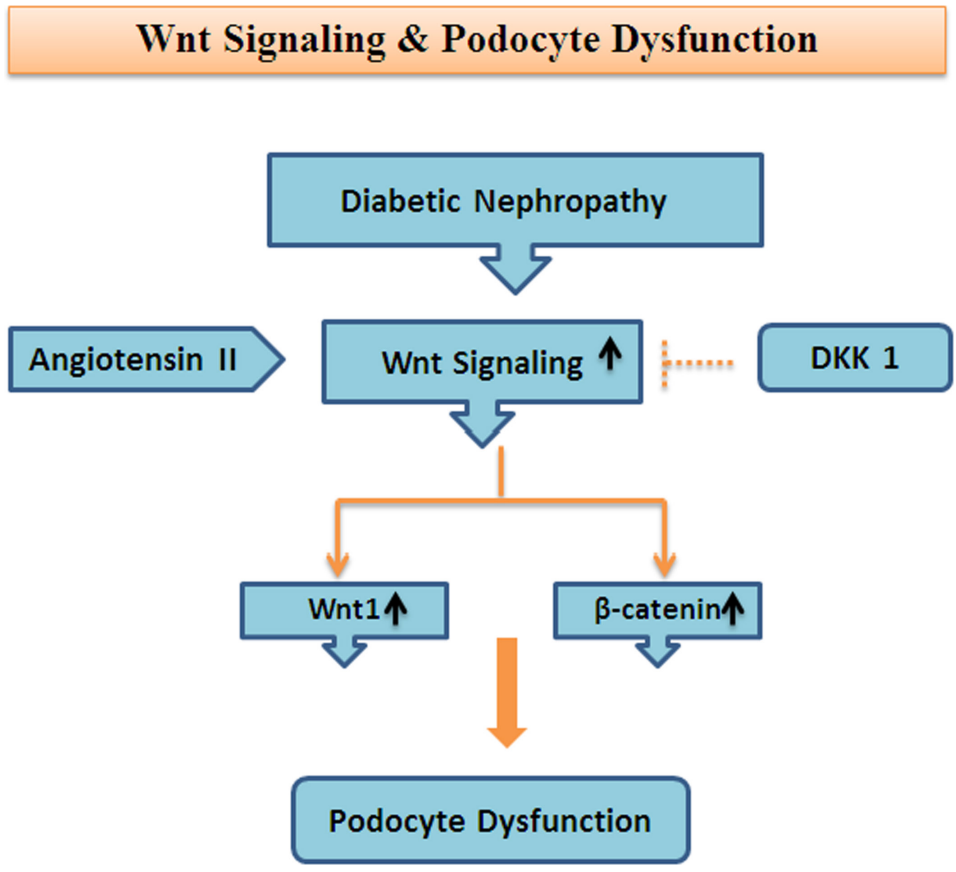

Figure 4 Wnt/β-catenin signaling in podocytes—suggested role in diabetic nephropathy.

small interfering RNA (siRNA) mitigated Ang II-induced podocyte injury. Furthermore, Ang II could also activate phosphorylation of calmodulin-dependent protein kinase (CaMK) II and cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) in podocytes. Thus, blockade of CaMK II/CREB pathway by either CK59, a CaMK II inhibitor, or CREB siRNA could significantly inhibit Ang II-induced Wnt/β-catenin signaling and ameliorate podocyte damage.[48] Kato *et al* demonstrated that Wnt/Ctnnb1 (β-catenin) signaling in podocytes plays a crucial role in integrating cell adhesion, motility, cell death, and differentiation. Interestingly, they found that Ctnnb1 deletion in cultured podocytes augmented expression of podocyte differentiation markers and podocyte motility but the cells were more susceptible to apoptosis. Their findings indicate that balanced expression of Ctnnb1 is essential for maintenance of glomerular filtration barrier.[36] Upregulation of Wnt1 and activation of β-catenin in podocytes have also been shown to suppress nephrin expression and induce Snail, a transcription factor which is involved in EMT of podocytes in DN.[1 6] Thus, Wnt/β-catenin signaling indeed plays a crucial role in podocyte injury and proteinuria during DN.

To summarize, in uncontrolled and long-standing diabetes, the Wnt/β-catenin signaling system is upregulated by high glucose levels and participates in MC apoptosis and EMT of podocytes and tubular epithelial cells that contribute to the development and progression of DN.

EFFECTS OF POTENTIAL THERAPEUTIC AGENTS IN DN MAY BE MEDIATED THROUGH WNT/B-CATENIN SIGNALING

Since Wnt/β-catenin signaling contributes to podocyte injury and loss, a major feature of DN, the concept of drugs targeting podocytes has been proposed in therapy of DN. Although specific drugs targeting particularly podocytes are not currently available, multiple therapeutic agents used for treatment of glomerular diseases have been investigated for their effects on Wnt signaling in podocytes.

ACE/angiotensin II-receptor blocker Inhibitors

Therapy with ACE inhibitors and angiotensin II-receptor blockers (ARB) result in significant improvement in systemic blood pressure, renal hemodynamics, and proteinuria in renal diseases. Angiotensin II is a regulator of glomerular capillary pressure which induces podocyte injury and ultimately leads to glomerulosclerosis. Thus, inhibition of angiotensin II production and/or receptor binding is an effective strategy in several kidney disorders. Angiotensin II also upregulates Wnt/β-catenin signaling pathway while angiotensin blockers attenuate angiotensin-II-induced podocyte damage in vitro and in vivo. ACE has also been shown to cause glomerular redistribution of Zona Occluden-s1 (ZO-1), a tight junction protein in

slit diaphragm of podocytes related to the development of proteinuria. Perindopril, an ACE inhibitor, has been shown to reduce foot process effacement in animal models of DN along with restoration of slit diaphragm proteins such as nephrin expression.[49] A recent study using 2K1C Goldblatt hypertensive rats tested the hypothesis that lisinopril, another ACE inhibitor, could ameliorate renal fibrosis by inhibiting β-catenin-dependent signaling pathway. It was found that after treatment with lisinopril, levels of β-catenin, p-Ser9-GSK-3β, and the β-catenin-dependent gene products in the kidney were reduced supporting the hypothesis.[50]

Vitamin D analogs

Vitamin D deficiency is a common problem in chronic kidney disease (CKD) even at the early stages, leading to bone and mineral disorders in such patients. Vitamin D analogs could reduce proteinuria in patients with CKD presumably by targeting the podocyte functions. Vitamin D has been shown to inhibit podocyte injury and loss, by activating expression of slit diaphragm proteins and maintaining integrity of glomerular filtration barrier. Vitamin D ameliorates podocyte injury by blocking pathways such as the renin-angiotensin system, Wnt/β-catenin pathway and pro-apoptotic pathway.[51] Zhang *et al* demonstrated that treatment with vitamin D analog doxercalciferol reduced albuminuria and glomerulosclerosis in STZ-induced diabetic mice (type 1 diabetes). Moreover, it also decreased podocyte foot process effacement along with the loss of WT1-positive podocytes and slit diaphragm proteins such as nephrin, Neph-1, ZO-1, fatty acid transferase (FAT-1), and actinin-4. The vitamin D analog when administered in combination with renin angiotensin system (RAS) (such as losartan) had augmented therapeutic efficacy suggesting that the mechanisms of the combination therapy entail more than simple inhibition of renin angiotensin system in the kidney.[52]

He *et al* recently reported that paricalcitol treatment in adriamycin-induced nephropathy in mice restored slit diaphragm-associated proteins (which were downregulated on adriamycin administration) indicating preservation of podocyte integrity by paricalcitol. The authors also reported inhibition of multiple Wnts, including Wnt4, Wnt7a, Wnt7b, and Wnt10a but not Wnt3 expression in renal tissue by paricalcitol.[53]

Protein kinase C inhibitors

Protein kinase C (PKC), an intracellular signaling molecule, is activated by high glucose levels and participates in the development of diabetic complications. However, several experimental and clinical studies have reported that the inhibition of PKC may delay/halt the progression of diabetic complications.[54] Calphostin C, a PKC inhibitor, was shown to decrease vascular endothelial growth factor expression in rat MCs in culture.[55] Another PKC inhibitor ruboxistaurin decreased albuminuria and maintained estimated glomerular filtration rate in patients with type 2 diabetes and nephropathy.[56] PKC is also known to cause Wnt-induced β-catenin stabilization and its downstream gene expression. Chen *et al* showed that calphostin C, a PKC inhibitor, suppressed Wnt induced potentiation of β-catenin accumulation.[57]

Thus, the therapeutic potential of many currently used and experimental drugs seem to exert their salutary effects at least in part by inhibiting the Wnt/β-catenin pathway, which is activated in DN.

CONCLUSIONS

While DN is the leading cause of renal failure needing renal replacement therapy in most parts of the world, understanding its pathogenesis remains unclear and challenging. Hemodynamic and metabolic factors that initiate renal injury in diabetes result in early changes in the structure and function of podocytes which cascade into glomerulosclerosis and nephronal loss through activation of growth factors and cytokines. Specifically, the effects on Wnt/β-catenin signaling plays a major role in mediating the podocyte dysfunction resulting in podocytopenia, Currently available therapeutic options in DN seem to exert their salutary effects through modulating Wnt signaling in podocytes. Emerging data on the role of Wnt signaling pathways in DN underscore its significance in pathogenesis and warrant more investigations to potentially develop novel therapeutic strategies targeting Wnt signaling.

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