

Podocyte Injury in Diabetic Nephropathy

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ABSTRACT

Diabetic nephropathy (DN) is a leading cause of end-stage renal disease throughout the world. It is well recognized that podocyte injury plays a critical role in the development and progression of DN. There are many breakthrough in the study of podocyte injury, such as podocyte apoptosis, epithelial-mesenchymal transition (EMT), autophagy and so on. In this review, we discuss pathogenesis of DN, highlight the cause of podocyte injury in DN, which would provide new insight into the prevention and treatment of DN.

Key words: Diabetic nephropathy; Podocyte injury; Apoptosis; Epithelial-mesenchymal transition; Autophagy

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INTRODUCTION

Diabetic nephropathy (DN) is one of the most common chronic serious complications of diabetes mellitus (DM) and the leading cause of end-stage renal disease. The global prevalence of DM has been increasing, and it is estimated that the people with DM worldwide will increase from 382 million in 2013 to 592 million by 2035, according to the International Diabetes Federation^[1]. Moreover, about 15%-25% of type 1 DM and 30%-40% of type 2 DM patients suffer DN^[2]. In this regard, it is extremely significant to study the pathogenesis and progressive mechanism of DN and try to delay its progression. However, the pathogenesis of DN remains fully elucidated. At present, many studies show that the development of DN is the results of interaction among genetics; hemodynamics, as well as metabolic factors. Furthermore, recent researches have showed podocyte also plays a critical role in DN progression. In this review, we summarize the present recognitions on the pathogenesis of DN, and highlight the relationship between podocyte injury and DN, which may help us to prevent and treat DN.

PATHOGENESIS OF DN

RAAS and DN

Renin-angiotensin-aldosterone system (RAAS) was uncovered more than 100 years ago; traditional RAAS includes rennin, angiotensin, aldosterone and related receptors, which maintains the hemodynamic stability and metabolic homeostasis of water and electrolytes, as well as blood pressure. RAAS is divided into systemic and local action according to action targets, and the kidney has complete RAAS system components. RAAS over-activation (especially in kidney) plays an important role in DN, in which Ang II is more critical, and elevation of Ang II in DN leads to hemodynamics disorder, especially renal hyperfiltration, hypertension and hypertransfusion in glomeruli. Ang II can promote proteinuria,

meanwhile, TGF- β induced by Ang II can damage podocytes, and exacerbate proteinuria^{13,41}. In recent years, angiotensin converting enzyme 2 (ACE2) is recognized as an important part of RAAS, and is more highlighted in the pathogenesis of DN. The researches showed that ACE2 expresses in podocyte and mesangial cells, but not in glomerular endothelial cells. ACE2 can physiologically protect the kidney by transforming Ang II to Ang-(1-7) and Ang I to Ang-(1-9)⁵¹. Both animal experiments^{5,61} and human researches^{17,81} showed that ACE2 expression was decreased in kidney of DN, and Ang II degeneration decreased, thereafter, accumulated Ang II in kidney jeopardized podocyte and induced proteinuria by means of hemodynamic and non-hemodynamic mechanism⁹¹. ACE2 inhibitor or knock-out of ACE2 exacerbated the progression of DN, which further indicated that ACE2 decrease played an important role in the development of DN^{5,10-121}. Wang *et al*¹³¹ found that ACE2 excreted in urine was positively related to proteinuria, in this regard, ACE2 has the potential to become a latent biomarker for DN. The role of RAAS on DN was gradually unveiled with the development of recognition to RAAS. In 2002, prorenin receptor (PRR) was first discovered and found it expressed in kidney and other organs. In kidney, PRR expressed on both mesangial cells and podocytes, and could work by bidding with prorenin and renin. The binding ability to prorenin of PRR is 2-3 fold higher than its bide to renin¹⁴¹.

Research showed that prorenin binds to PRR to transform into renin with catalytic activity by means of change of molecular conformation and exposure to active site, then Ang II generates and participates in the pathogenesis of DN (Ang II dependent pathway). On the other hand, prorenin binding to PRR can directly activate intracellular MAPK (Ang II independent pathway), thereafter increase mesangial TGF- β level and matrix protein accumulation¹⁴¹. Experiments found that PRR expression increase in both type 1^{15,161} and type 2¹⁷¹ DN animal models, and PRR blockers can inhibit its activity, and postpone DN progression, which indicate PRR play an significant role on the development of DN¹⁸¹. Recent study found that PRR induced increases of inflammatory factor TNF- α and IL-1 β expression, which participate in the development of DN. PRR blockers can decrease both TNF- α and IL-1 β expression, and reduce proteinuria as well¹⁹¹. PRR is expected to become a new target for the treatment of DN by intensive study its role on the pathogenesis of DN.

OXIDATIVE STRESS AND DN

Other than hemodynamic factor, metabolic disorders also play a critical role on pathogenesis of DN. Prolonged exposure to hyperglycemia is an initiating factor for DN. In the later stages of diabetes, even if blood glucose control is satisfactory, a series of subsequent reactions caused by hyperglycemia still lead to DN progression because of so-called “glucose memory”. The ADVANCE study indicated the role of hyperglycemia on pathogenesis in DN, and intensive blood glucose control reduced the risk of at least 10% to endpoint events, and relative risk of 21% to DN²⁰¹. Intracellular glucose dysmetabolism under the situation of hyperglycemia activated a series of signal pathways; generated ROS products, TGF- β , advanced glycosylation end products (AGEs), as well as MCP-1, eventually lead to a series of diabetic complications. Glycosamine intermediate product transformed from fructose-6 -phosphate can further generate ROS and TGF- β after hexosamine pathway activation. DAG-PKC signal pathway can also generate inflammatory factors, such as ROS and TGF- β . In setting of DM, AGEs accumulate in the body, and when AGEs bind to AGE receptors, increased

ROS will lead to oxidative stress and inflammation factors release, all of these changes will eventually lead to renal injury. Meanwhile, hyperglycemia activate polyol metabolic pathway and consume a lot of reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH). NADPH is important to maintain the normal level of glutathione (GSH) that plays a vital role in ROS clearance in cells, in this regard, reduction of NADPH give rise to oxidative stress by means of GSH reduction and ROS accumulation.

Giacco *et al*²¹¹ comprehensively analyzed the roles of various metabolic factors on complications of DM and found that oxidative stress is the common pathway for DN. Normally, production and clearance of ROS are in balance, however, ROS increase or antioxidant decrease induced by various factors in DM setting can contribute to oxidative stress^{22,231}. Oxidative stress play a critical role in the pathogenesis of DN, increased ROS can inhibit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by activating poly ADP-ribose polymerase (PARP), then cause glycolysis dysfunction, eventually lead to complications of DM due to various metabolic pathway activation^{21,241}.

Recent research showed that RAAS activation and oxidative stress were not isolated in the pathogenesis of DN, they cross-talk each other and together contribute to progression of DN^{25,261}. Both ACEI and ARB can protect the kidney by not only lowering blood pressure, but also inhibiting AGEs production, which confirms the cross-talk between hemodynamics and metabolic factors^{27,281}. ROS in body mainly derives from NADPH oxidase²⁹¹, in the states of disease, metabolic disorder produce excessive ROS, moreover, in early stage of DM, Ang II also results in the generation of excessive ROS by inducing NADPH oxidase³⁰¹.

ROLE OF PODOCYTE IN DN

Glomerular filtration barrier (GFB) includes charge barrier and size barrier. There are 3 layers, including endothelial cell, glomerular basement membrane and podocyte, the latter is the critical part of GFB. Podocyte derives from mesenchyme cell during fetal development, and loses its proliferative ability after maturity. Podocyte is consisted of cell body, primary processes and foot processes. Combined with endothelia cell and glomerular basement membrane (GBM), podocytes maintain normal glomerular filtration function by attaching on GBM with foot processes, and the injury of podocyte structure and function would lead to proteinuria. Various studies showed podocytopathy is a cardinal issue for pathogenesis of DN³¹⁻³⁵¹, and podocyte injuries include cellular hypertrophy, foot processes effacement, and podocyte detachment. Decreased number of podocyte gives rise to glomerular capillary loop collapse, GBM exposure, adhesion to Bowman’s capture; finally result in glomerulosclerosis³⁶¹.

PODOCYTE HYPERTROPHY

Cell hypertrophy is a common kind of pathological alteration in the body, and it means the cell cycle is stagnated in set point of G1/S phase, fail to synthesize DNA with the increase of protein synthesis. That is a common adaptive pathological process, under this condition, the cell volume increases, but the number is unchanged³⁷¹. In the setting of DM, hyperglycemia and mechanical stress acting on podocyte, leading to increase podocyte volume and inhibit podocyte proliferation by enhancing the expression of cyclin dependent kinase inhibitor P27^{Kip}^{38, 391}. Xu *et al*⁴⁰¹ found that Ang II involved in podocyte enlargement process, and angiotensin II receptor blocker

(ARB) could inhibit p27^{Kip1} expression. AGEs also play an important role in podocyte injury in the early stage of DN^[41]. Hyperfiltration, hypertension and hyperperfusion in glomeruli in DM situation lead to glomerular hypertrophy, podocyte adaptive hypertrophy as well in order to maintain normal glomerular structure and function. However, podocyte density decrease to a certain extent will give rise to podocyte detachment^[42]. Recently, Herbach *et al*^[43] found that podocyte hypertrophy was earlier than glomeruli enlargement in the development of DN, indicating that podocyte hypertrophy is critical for DN.

PODOCYTE LOSSES

In addition to podocyte hypertrophy in DN, attention to podocyte loss was also paid by many studies in earlier time. Podocyte loss was very common in both type 1 and type 2 DN patients, and there was positive correlation between podocyte loss and proteinuria progression^[44-47]. Previous studies showed that podocyte density in glomeruli was more valuable than podocyte loss to predict progression of DN^[48,49]. The other reasons for podocyte loss include apoptosis, detachment, as well as decrease of podocyte regeneration.

Podocyte apoptosis

Podocyte apoptosis was verified as an important contributing reason to pathogenesis of DN in most experiments. Susztak found ROS in the setting of hyperglycemia could activate both p38MAPK and caspase-3 not only *in vitro*, but *in vivo* of animal model, eventually cause proteinuria^[50]. AGEs can also activate FOXO4 transcription factor through p38MAPK signal pathway, then lead to podocyte apoptosis^[51]. Increased ROS due to up-regulation of 20-HETE and NADPH oxidase by cytochrome in hyperglycemia situation can also cause podocyte apoptosis^[52]. Recently, it has been found that Notch1 signal dependent activation of p53, rather than p38MAPK, was involved in the occurrence of podocyte apoptosis, which is considered as a new way of podocyte apoptosis^[53]. Further study indicated that Notch expression of podocyte correlated with proteinuria and glomerular sclerosis, so Notch activations may be the general pathogenesis of acquired kidney diseases, including DN^[54].

Normally, proapoptotic and anti-apoptotic signal pathways exist simultaneously in body and keep dynamic equilibrium, and maintain stability of internal environment. Decreased activity of anti-apoptotic signal is a critical reason for podocyte apoptosis. PI3K/AKT pathway in podocyte can inhibit podocyte apoptosis, experiments showed that AKT phosphorylation reduction could cause increase of podocyte apoptosis of db/db mice^[55]. Various reasons such as hyperglycemia, AGEs, oxidative stress, Ang II, as well as TGF- β act on podocyte concomitantly in DN, then lead to podocyte loss by activating apoptotic pathway and down-regulating anti-apoptosis signal pathway.

Podocyte detachment

A research on urinalysis of 50 patients with type 2 diabetes showed that urinary podocytes were detected in 53% patients with microalbuminuria and 80% with macroalbuminuria, whereas absent in healthy controls, diabetic patients with normoalbuminuria and diabetic patients with chronic renal failure^[56]. Further, some living but not apoptotic podocytes were found in the urine of animal models and patients with DN, demonstrating that podocyte detachment is another reason for podocyte loss^[57,58]. Some researchers considered that podocyte in urine occurred earlier than proteinuria to reflect kidney injury, in this regard; podocyte in urine was a biomarker in

early stage of DN^[56,59].

Podocyte structure and function is maintained by attaching GBM through $\alpha3\beta1$ integrin and deglycosylation, cytoskeleton rearrangement, decrease of surface negative charge and integrin expression give rise to podocyte detachment^[37]. It is showed that decrease of $\alpha3\beta1$ integrin expression between podocyte and GBM in hyperglycemia is an important reason for podocyte detachment^[60]. In addition, mechanical stress and TGF- β reduce podocyte adhesion ability by down-regulating $\alpha3\beta1$ integrin expression^[61]. Chen *et al* found that $\alpha3\beta1$ integrin expression and podocyte adhesion ability decreased in podocyte cultured with high glucose milieu, and integrin linked kinase (ILK) expression increased; however, astragalin IV can inhibit ILK expression and up-regulate $\alpha3\beta1$ integrin, and improve podocyte adhesion ability^[62]. Han *et al* found that podocyte phenotype altered and its adhesion capability decreased with both ILK and $\beta1$ integrin expression up-regulation under the stimulation of either high glucose solute or Ang II, which are improved by ARB treatment^[63]. The reason why podocyte detachment with different integrin level still remains to be elucidated.

Epithelial-mesenchymal transition (EMT)

EMT is another pathogenesis for podocyte loss in DN. During the process of EMT, podocytes loss some normal epithelial markers but acquire mesenchymal markers. Li *et al* found that TGF- β could induce podocyte phenotype change by downregulating the expression of epithelial biomarkers such as nephrin, ZO-1, P-cadherin, whereas upregulating the expression of mesenchymal markers such as desmin, FSP-1 (fibroblast-specific protein-1), MMP-9 (matrix metalloproteinase-9) and fibronectin^[64]. Recently, some questions have been raised about the origin of the detachment of podocytes in urine, which is derived from visceral epithelial cells or parietal epithelial cells^[65]. Yamaguchi *et al* found that, in urinary sediment, about 86% of podocyte expressed FSP-1, in which 95% were without apoptosis^[66]. FSP-1 was also found in kidney tissue specimen, the level of FSP-1 correlated with proteinuria, podocyte loss, as well as severity of kidney pathology, these findings firmly verified that EMT is one of the reasons for podocyte loss. Our previous studies *in vivo*^[67] and *in vitro*^[68,69] have also demonstrated that podocytes undergo EMT in DN. Thus, how to protect podocyte from EMT is an important issue in the field of DN research.

Lack of podocyte proliferation

Cell growth depends on the normal cell cycle; and the latter is tightly regulated by the cell cycle regulatory proteins in the cell nucleus. Positive regulation will promote cell cycle; however, negative regulation will inhibit cell proliferation. Positive regulative proteins of cell cycle include cyclin and cyclin dependent kinase (CDK), both of which have activity after binding together to form complex. Cyclin dependent kinase inhibitor (CDKI) can specifically bind with CDK, and then inhibit cell proliferation. There are 2 types of CDKI, one is INK family including P15, P16, P18 and P19, the other is CIP/KIP family including P21, P27 and P57, the latter is most studied^[37].

In response to various injury stresses, the lack of proliferation is one of the reasons which lead to the loss of podocyte^[37]. Podocyte is a kind of terminally differentiated cell. In normal resting state, podocyte expresses P27 and P57, but not P21^[70]. It is reported that podocyte is lack of proliferation in membranous nephropathy (MN), DN and typical focal segmental glomerulosclerosis (FSGS), whereas it is capable of proliferating in HIV associated nephropathy, crescentic nephritis and cellular proliferating FSGS. The reason why different patterns of podocyte mentioned above is experimentally studied due

to abnormal expressions of P27, P57 and P21. In the setting of DN, podocyte stimulated by high glucose overexpresses p27^{Kip1}, which can inhibit podocyte proliferation, and attenuation of p21^{Kip1} gene would ameliorate podocyte injury in DN^[71,72].

Another important kinase that plays a key role in the regulation of podocyte biology is Cdk5, which is activated and regulated by p35 and cyclin I and has been shown to control podocyte survival. The Cdk5 probably stabilizes the prosurvival protein Bcl2 and protects podocytes against apoptosis *in vitro* and *in vivo*^[73,74].

Podocyte autophagy

Autophagy is regarded as a major catabolic process in a cell that degrades and recycles macromolecules and damaged organelles to maintain intracellular homeostasis and cell integrity^[75]. In the kidney, emerging evidence shows that autophagy plays a critical role in the maintenance of kidney homeostasis and disease pathogenesis^[76,77]. In normal condition, our previous study and others indicated that podocytes have a high level of basal autophagy, which are important for their maintenance of cellular homeostasis. Importantly, impaired podocyte autophagy exacerbates proteinuria in DN^[75-77]. Several studies have shown that hyperactivation of the mTOR pathway plays a pivotal role and is associated with podocyte injury in DN. It is reported that mTOR plays a major role in the negative regulation of autophagy. In contrast, AMPK is a positive regulator of autophagy, furthermore, it can crosstalk with the mTORC1 signal during multiple steps of autophagy regulation. Sirt1, one of the members of sirtuins, has been shown to induce autophagy and may contribute to a renoprotective effects in DN. Thus, activation of autophagy may be a therapeutic option for the prevention and treatment of DN^[78,79]. Our study indicated that treatment with the mTORC1 inhibitor rapamycin restored autophagic activity and attenuated hyperglycemia-induced podocyte injury^[80]. Furthermore, autophagy inhibition induced podocyte apoptosis, which provide a new insight into the interaction between autophagy and apoptosis in the injury of podocyte. There was a report that autophagy inhibition induced podocyte apoptosis by activating the pro-apoptotic pathway of endoplasmic reticulum stress^[81].

PODOCYTE RELATED PROTEINS

It is demonstrated that podocyte is composed of three parts according to its cellular structure: the apical membrane domain, the junctional domain, and the basal domain^[82]. The apical membrane domain consists of podocalyxin, podoplanin and podocin, which carry negative charges and acts as a charge barrier to prevents albumin filtered from glomeruli. The junctional domain including nephrin, podocin, ZO-1 and CD2AP, which were important parts of slit diaphragm (SD) and acts as mechanical barrier. The basal domain mainly consists of α 3 β 1 integrin and dystroglycans, which are mainly in charge of adhesion between podocyte and GBM to prevent podocyte detachment.

SD is a key structure of selective filtration barrier of kidney, acts as a bridge between foot processes. SD consists of a series of protein complexes in the extracellular space of podocytes. Interaction of these protein complexes can not only maintain normal podocyte structure and biological function, but also intimately relate to proteinuria. Nephrin is an important part of SD, also well studied. Nephrin was discovered in 1999 by Tryggvason, it is one of immunoglobulin super family and coded by NPHS1 gene with molecular weight 180kd. Nephrin is a transmembrane protein with cytoplasmic domain and plasmalemma domain^[82]. Substantial evidence indicates that

nephrin-CD2AP-podocin complex is a key functional unit to rivet SD on cytoskeleton of podocyte cytotactin so as to maintain normal glomerular filtration function^[83-87]. One of cross-sectional studies on DM showed that 17% of microalbuminuria patients and 28% of heavy proteinuria patients were detected nephrin in urine, but there was no nephrin in normal control group^[88]. Meanwhile, nephrin expression decreases in both types 1 and type 2 DN, and correlate with progression of renal diseases^[89-93]. Both ACEI and ARB can prevent nephrin reduction, however, calcium channel blocker and endothelin receptor antagonist can not, which indicate that Ang II is one of the pathogenesis in nephrin reduction^[94-97].

Coward *et al*^[98] found that podocyte was a kind of insulin sensitive cells, and was the insulin target. Nephrin plays an important role on uptaking of insulin and glucose metabolite in podocyte under the setting of high glucose^[99]. Compared with normal nephrin expression cells, podocyte without nephrin expression had no response to insulin, and its capability of uptaking glucose induced by insulin apparently reduced, however, podocyte without nephrin expression transfected with nephrin gene could revert glucose uptake capability induced by insulin^[99]. Further study showed that podocyte insulin specific signal defect participated in early stage of occurrence and development of DN^[99].

In addition to nephrin, expression of both P-cadherin and ZO-1 decreased prominently in DN^[100,101]. Not all podocyte related proteins reduce; CD2AP and podocin are intact in DN^[90,91]. Further studies needed to elucidate the interrelationship between nephrin and other SD related proteins.

TRANSIENT RECEPTOR POTENTIAL CATION CHANNEL 6 (TRPC6) AND PODOCYTE

Podocytes injury is considered a major contributor to the development and progression of DN. In normal conditions, the physiological function of podocytes is dependent on their handling capacity of intracellular calcium. Excessive calcium influx in podocytes may result in the effacement of foot processes, apoptosis, and subsequent glomeruli damage. One of the main proteins responsible for calcium flux in podocytes is TRPC6, which is a channel in glomerular slit diaphragm^[102]. Compelling data have indicated that this channel play a critical role in various kidney diseases, including DN^[103-105]. A lot of efforts have been done to unraveling the pathogenesis of TRPC6-mediated podocyte injury in DN. It is indicated that TRPC6 is located on the podocyte membrane, where it is integrated into a signaling complex that interacts with nephrin and other podocyte related proteins^[102,106,107]. Ilatovskaya *et al* found that angiotensin II-dependent activation of TRPC6 is implicated in type 1 DN^[105]. Liu *et al* found that high glucose induced apoptosis in podocytes which express TRPC6, but not in TRPC6 knockdown podocytes, furthermore, the cause of apoptosis was due to the elevation of ROS by stimulating TRPC6 in high glucose condition^[108]. Another study demonstrated that high glucose modified TRPC6 channels via increased oxidative stress and syndecan-4 in human podocytes^[109].

CONCLUSION

Up to now, metabolic disorders, hemodynamic abnormalities and different gene background are implicated in the pathogenesis of DN. Most of these factors firstly cause podocyte injury, destroy filtration barrier, and then give rise to proteinuria, the latter lead to tubular-interstitial inflammation, eventually kidney fibrosis. According to the pathogenesis of DN, it is beneficial to prevent DN clinically by

correcting metabolic and hemodynamic disorder, especially protect the podocyte as early as possible.

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