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Review

The Physiopathologic Roles of Calcium Signaling in Podocytes

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Abstract

Calcium (Ca^{2+}) plays a critical role in podocyte function. The Ca^{2+}-sensitive receptors on the cell surface can sense changes in Ca^{2+} concentration, and Ca^{2+} flow into podocytes, after activation of Ca^{2+} channels (such as transient receptor potential canonical (TRPC) channels and N-type calcium channels) by different stimuli. In addition, the type 2 ryanodine receptor (RyR2) and the voltage-dependent anion channel 1 (VDAC1) on mitochondrial store-operated calcium channels (SOCs) on the endoplasmic reticulum maintain the Ca^{2+} homeostasis of the organelle. Ca^{2+} signaling is transmitted through multiple downstream signaling pathways and participates in the morphogenesis, structural maintenance, and survival of podocytes. When Ca^{2+} is dysregulated, it leads to the occurrence and progression of various diseases, such as focal segmental glomerulosclerosis, diabetic kidney disease, lupus nephritis, transplant glomerulopathy, and hypertensive renal injury. Ca^{2+} signaling is a promising therapeutic target for podocyte-related diseases. This review first summarizes the role of Ca^{2+} sensing, Ca^{2+} channels, and different Ca^{2+}-signaling pathways in the biological functions of podocytes, then, explores the status of Ca^{2+} signaling in different podocyte-related diseases and its advances as a therapeutic target.

Keywords: calcium; calcium signaling; disease; podocyte; target

1. Introduction

Podocytes are terminally differentiated epithelial cells located on the medial side of the Bowman's capsule [1]. The nucleus of a podocyte is located in the center of the cell, which has a large cell body. Foot processes (FPs) are irregular protrusions extending from the cell body [2]. The basal area of the FP adheres to the glomerular basement membrane (GBM). In addition, adhesion molecules and connexins exist between individual FPs, thereby forming a porous structure known as the slit diaphragm (SD) [3]. The unique morphological and structural characteristics of podocytes are essential for the maintenance of the glomerular filtration barrier function. In a variety of pathologic conditions, the structure and morphology of podocytes are affected, and the glomerular filtration barrier is impaired, leading to the occurrence of podocyte-related diseases [4].

Acting as an important second messenger for cells, calcium (Ca^{2+}) participates in and regulates various cellular activities, including cell apoptosis and proliferation [5]. Cellular and extracellular Ca^{2+} are in dynamic balance, and calcium-sensing receptors (CaSRs) can sense and regulate changes in Ca^{2+} concentrations [6]. At the same time, the strict regulation of intracellular Ca^{2+} is inseparable from the expression and activity of various channel proteins. Ca^{2+} signals transmitted by channel proteins are essential for maintaining podocyte function [7]. Ca^{2+} signaling is required for podocyte morphogenesis and FP formation [8]. Normal Ca^{2+} signaling can maintain the structure of podocytes by stabilizing cytoskeletal proteins [9,10]. In addition, downstream signaling mediated by Ca^{2+} can regulate the autophagy balance in podocytes and participate in cell survival [11]. The function of podocytes is critically dependent on the processing of intracellular Ca^{2+} signaling.

In the pathological states of the podocyte, enhanced Ca^{2+} signaling in podocytes is observed [12]. Changes in Ca^{2+} transport and signaling are related to the occurrence and development of various podocyte-related diseases [9,13,14]. Further understanding of the status of Ca^{2+} signaling in podocytes could provide promising insights for the treatment of podocyte-related diseases. This review summarizes the current studies on Ca^{2+} sensing, Ca^{2+} channels, and the Ca^{2+}-signaling pathway in podocytes, as well as the status and targets of the Ca^{2+}-signaling pathway in podocyte-related diseases.

2. Ca^{2+} Sensing and Transport in Podocytes

2.1 Ca^{2+} Sensing

In mammals, Ca^{2+} homeostasis is mainly mediated by CaSRs [6]. In 2008, Piecha *et al.* [15] first described the presence of CaSRs in podocytes. The podocyte-specific CaSR knockout model suggested that the CaSR represented an important receptor in the maintenance of glomerular filtration barrier function [16].

A CaSR is a G-protein-coupled receptor (GPCR), which encodes a gene that is located on chromosome 3 in

Table 1. Subcellular localization and function of Ca^{2+} channels in podocytes.

Calcium channels		Subcellular localization	Function	Reference
TRPCs	TRPC1 TRPC4	Unclear	Promotes Ca^{2+} influx	[20]
	TRPC3 TRPC5 TRPC6	Plasma membrane and organelle membranes, such as ER, Golgi, lysosomes, etc.	Promotes Ca^{2+} influx	[21,24,31] [22,24,32] [22–24]
SOCs	STIM1 ORAI1	ER Plasma membrane	Sensor for luminal Ca^{2+} concentration in ER Promotes an influx of Ca^{2+} into the cytoplasm	[25–27]
RyR 2		ER	Calcium release from ER	[28]
VDAC 1		Mitochondria	Transports Ca^{2+} into the mitochondria	[29]
Cav2.2		Plasma membrane	Promotes Ca^{2+} influx	[30]

Abbreviations: TRPCs, transient receptor potential canonical channels; SOCs, store-operated calcium channels; STIM1, stromal-interacting molecule 1; ORAI1, Ca^{2+}-release-activated Ca^{2+} channel 1; RyR 2, type 2 ryanodine receptor; VDAC 1, voltage-dependent anion channel 1; Cav2.2, N-type calcium channel; ER, endoplasmic reticulum.

humans. CaSRs can be activated by type I and type II agonists. Type I agonists include divalent ions, such as Ca^{2+} and magnesium, β-amyloid peptide, and polycation, which directly stimulate the CaSR. Type II agonists are a class of regulatory substances, including spermidine, spermine, aromatic amino acid residues, extracellular pH, and ionic strength. In the presence of extracellular Ca^{2+}, type II agonists increase the Ca^{2+} affinity of the CaSR by allostery [6]. The CaSR activates the downstream signaling cascade through coupling with heterotrimeric guanine nucleotide-binding proteins (G proteins), such as the Gi/o, G12/13, and Gq/11 families [17]. An increase in Ca^{2+} stimulates the activity of Gq/11 and phospholipase C (PLC), inducing the accumulation of inositol 1,4,5 triphosphates (IP3). Subsequently, IP3 attaches to the endoplasmic reticulum (ER), via the IP3 receptor, and induces Ca^{2+} influx via the storage manipulation channel located on the plasma membrane, which results in Ca^{2+} being released from the intracellular stores [18]. In podocytes, the activated CaSR can promote TRPC6-dependent Ca^{2+} entry [19].

The CaSR-mediated Ca^{2+} balance of podocytes, which is key for podocyte normal function, has gradually become a research focus.

2.2 Ca^{2+} Channels

The transport of Ca^{2+} in podocytes is associated with multiple channel proteins (Table 1, Ref. [20–32]). TRPCs, which belong to the transient receptor potential (TRP) superfamily, are the most widely studied channel family in podocytes [5]. There are seven isoforms of the TRPC proteins, including TRPV1-7. TRPCs are "non-selective" cation channels that can promote Ca^{2+} influx into the cells [33]. Studies demonstrated that TRPC1, TRPC3, TRPC4, TRPC5, and TRPC6 are all expressed in podocytes [7,20–22], although only TPRC 3, TRPC5, and TRPC6 were shown to contribute to Ca^{2+} entry into the podocyte [23]. In podocytes, TRPCs can be activated by a variety of upstream signals, whereas Ca^{2+} stimulates the downstream Ca^{2+}-dependent signaling cascade [34–36]. Depending on their location in the cell, TRPCs are involved in different cellular physiological processes. In organelles such as the ER, Golgi, and lysosomes, their activation also promotes Ca^{2+} influx [24].

SOCs in the plasma membrane are triggered and activated by the depletion of Ca^{2+} from the ER. SOCs consist of a single-channel transmembrane protein, STIM1, in the ER, and a small plasma membrane protein, ORAI1 [25,26]. When Ca^{2+} is depleted in the ER, STIM1 is activated and aggregates in the vicinity of ER-plasma membrane junctions. STIM1 activates ORAI1, resulting in an influx of Ca^{2+} into the cytoplasm [27]. The expressions of STIM1 and ORAI1 were observed in mouse podocytes [37]. In addition, overexpression of STIM1 and ORAI1 increased the Ca^{2+} influx in podocytes [37].

Increased intracellular Ca^{2+} is a burden on organelles that may cause dysfunction [38]. RyR2 in the ER, as well as VDAC1 in the mitochondria, mediate Ca^{2+} transport in the organelles [28,29]. Researchers have mentioned the role of the RyR2/Ca^{2+}-release channel in ER–Ca^{2+} balance [28]. In podocytes, VDAC1 controls the Ca^{2+} transport into and out of the mitochondria, which is essential for mitochondrial function [29]. The N-type calcium channel, Cav2.2, in podocytes, as a Ca^{2+} transport channel, may be associated with the activation of the local renin-angiotensin system (RAS) [30].

These Ca^{2+} transport channels are important components of cells and organelles. Due to the different pathophysiological statuses, activity changes in these channels cause an imbalance in cellular Ca^{2+} homeostasis, which may relate to the pathogenesis of some podocyte-related diseases [39]. Thus, using calcium channels as therapeutic targets has the potential for podocyte-related diseases.

3. Role of Ca^{2+} Signaling in Podocyte Cell Biology and Physiology

Ca^{2+} signaling is necessary for multiple pathophysiological functions of podocytes. Recent studies have shown that Ca^{2+} signaling is involved in the morphology, architecture, and survival of podocytes. We now describe in detail the roles of Ca^{2+} signaling in different biological processes involving podocytes.

3.1 Podocyte Morphology

Podocytes are essential for the maintenance of the glomerular filtration barrier. Studies have shown that mutations in TRPC6 associated with nephrotic syndrome have led to the disruption of the filtration barrier through elevated cytoplasmic Ca^{2+} in podocytes [40,41]. The distribution of TRPC6 channels changes as podocyte differentiation matures [42]. This suggests that Ca^{2+} signaling may play a critical role in filtration-barrier formation. A study used transgenic zebrafish larvae and human kidney organoids to explore the role of Ca^{2+} signaling during podocyte development [8]. They found that Ca^{2+} signaling was active during podocyte differentiation, resulting from the triggering and release of intracellular Ca^{2+}. This Ca^{2+} influx may be mediated through PLCg/IP3, leading to podocyte-foot-process differentiation and glomerular-filtration-barrier formation. The role of Ca^{2+} signaling in podocyte morphogenesis cannot be ignored and deserves more attention.

3.2 Podocyte Architecture

The tight and complex interactions between the slit diaphragm, focal adhesion, and the actin cytoskeleton are essential for maintaining the normal structure and function of podocytes [43]. Slit diaphragm proteins, focal adhesion protein complexes, and actin cytoskeleton proteins play important roles in the precise organization and regulatory role of the actin cytoskeleton. Ca^{2+} signaling can have an impact on podocyte structure by affecting cytoskeletal proteins.

Podocin and nephrin are known to be critical SD proteins in maintaining podocyte structural stability [44]. Lu *et al*. [10] elucidated the role of TRPC6 in high glucose-induced podocin reduction. In their study, TRPC6 knockdown resulted in a diminution of the decreased podocin in podocytes, and a high level of glucose decreased podocin through activating TRPC6. This suggested that podocin can be mediated by TRPC6, the activation of which results in an increase in intracellular Ca^{2+}. However, the exact mechanism of action between TRPC6 and podocin remains unclear. Ca^{2+} is known to regulate protein degradation through ubiquitination [45], which could be an interesting focus for future research.

Bhargava *et al*. [9] reported that IgG from kidney transplant recipients with transplant glomerulopathy activated the calmodulin kinase IV (CaMK4)/glycogen synthase kinase-3 β (GSK3β) pathway. Phosphorylated GSK 3β stabilized nephrin transcriptional repressor SNAIL, which caused a reduction in nephrin expression [9]. Their group also demonstrated that a reduction in nephrin expression, induced by IgG from lupus nephritis, was associated with the activation of CaMK4 [46]. Although Ca^{2+} channels were not mentioned in these studies, IgG, which has been produced in the pathological state, has been shown to trigger TRPCs in several studies [47,48]. This may also be the mechanism through which IgG activates CaMK4 in kidney diseases.

Several signaling pathways, including CaMK4, play essential roles in actin remodeling after an increase in Ca^{2+}. Synaptopodin, an actin-binding protein, regulates the integrity and motility of podocytes through the Rho family of small GTPases (including Rac1, Cdc42, and RhoA) [49]. The pCaMK4 phosphorylates scaffold protein 14-3-3, which leads to the release and degradation of synaptic foot proteins [50], by enhancing Rac1 expression and decreasing RhoA expression [50,51]. Rac1 promotes cell motility by forming lamellipodia, and RhoA inhibits cell motility by promoting the formation of contractile actin [52]. Insulin triggers store-operated Ca^{2+} entry, which leads to a decrease in synaptopodin via the Ca^{2+}/calcineurin pathway [9,10,53].

Talin-1 is a podocyte cytoskeleton-associated anchor protein and a specific target of calpain [54]. Studies have shown that aberrant Ca^{2+} signaling can be transmitted through the TRPC to activate downstream calpain and calcineurin, which then, exert effects on the actin cytoskeleton by reducing Talin-1 expression [43,55]. Farmer *et al*. [55] reported that TRPC6 can interact with calpain 1 and calpain 2 and maintain calpain localization at the membrane. This physical interaction is significant for cytoskeletal stability in podocytes, where the actin cytoskeleton forms a highly dynamic contractile state, which is critical for the integrity of the foot processes in podocytes [56]. The CaSR is directly connected to the cytoskeleton by binding to the thin filament protein A. Oh *et al*. [57] showed that CaSR activation increased the actin density and content in podocytes, and reduced the degradation of the actin cytoskeleton caused by PAN.

The biological aspects of Ca^{2+} signaling in podocytes cannot be ignored. Multiple pathological states cause podocyte structural changes via Ca^{2+} imbalance; thus, by regulating structure-related proteins, Ca^{2+} signaling also influences the mobility and stability of podocytes.

3.3 Podocyte Survival

The survival of podocytes is a complex physiological process associated with multiple factors, such as the integrity of the cytoskeleton and expression of various pro-survival genes, and autophagy [58–60]. The stability of the cytoskeleton underlies cell survival. Ca^{2+} signaling can affect podocyte survival by mediating the impairment of

the cytoskeleton in podocytes. Recent studies have shown that Ca^{2+} signaling also regulates the expression of multiple survival factors. Oh *et al*. [57] reported that the CaSR mediates the survival of podocytes through the MAPK pathway. The transcription factor, the cAMP-response element-binding protein (CREB) is known to be a pro-survival gene activator, while BAD and Bcl-xl are also pro-survival factors [57]. When the CaSR was activated, the expression of phosphorylated CREB, BAD, and Bcl-xl increased. Hence, an activated CaSR mediates the phosphorylation of the p90 RSK through ERK1/2, ultimately leading to the phosphorylation of CREB, the pro-survival gene activator [57].

In addition to the CaSR, the relationship between downstream signaling mediated by Ca^{2+} channels and cell survival should not be ignored. Autophagy is essential for the homeostasis and survival of podocytes. Calpain in podocytes can be activated by Ca^{2+}/calcineurin [54]. Bensaada *et al*. [61] found that the inhibition of calpain can maintain autophagy in podocytes during hypertension. The TRPC6 pathway also mediates autophagy in podocytes [62]. Ma *et al*. [11] reported that AngII can activate TRPC 6 and cause an increase in Ca^{2+} influx, which results in the activation of calmodulin-dependent protein kinase-β (CaMKKβ), which, when activated, promotes the formation of activated protein kinase (AMPK) and autophagy in podocytes. AngII-induced abnormal autophagy and damage in the podocyte can be alleviated following the inhibition of TRPC6-mediated Ca^{2+} influx or downstream Ca^{2+}-signaling pathways, such as calcineurin [11,63].

Stabilization of Ca^{2+} channels in multiple organelles is also essential for maintaining podocyte homeostasis. The mitochondrial Ca^{2+} unidirectional transporter (MCU) maintains mitochondrial Ca^{2+} homeostasis to ensure its normal function [64]. RyR2 in the ER is involved in ER–Ca^{2+} leakage as well as ER stress [28]. The trigger of SOCE in the ER also has a major impact on podocyte homeostasis [65].

4. Ca^{2+} Signaling in Podocyte-Related Diseases

As critical components of the glomerular filtration barrier, podocytes can modulate glomerular filtration function. Podocyte dysfunction is central to the pathophysiology of many common glomerular diseases, including diabetic kidney disease, glomerulonephritis, and genetic forms of nephrotic syndrome (NS) [66]. By intravital imaging of podocyte Ca^{2+}, one study suggested that the conduction of Ca^{2+} signaling was a key pathogenic mechanism in various podocyte-related diseases [12]. Ca^{2+} signaling is involved in various podocyte-related kidney diseases, including focal segmental glomerulosclerosis, diabetic kidney disease, lupus nephritis, transplant glomerulopathy, and hypertensive renal injury. Below we describe the mechanisms of Ca^{2+} signaling in various diseases.

4.1 Focal Segmental Glomerulosclerosis (FSGS)

FSGS is an important cause of end-stage renal failure (ESRD), characterized by podocyte loss and dysfunction. Most patients show steroid-resistant nephrotic syndrome (SRNS).

Several studies have shown that genetic FSGS is associated with mutations in the TRPC6 gene [39,67–69] (Fig. 1). Among the mutated genotypes, TRPC6 always showed a gained functional phenotype involving Ca^{2+}-mediated podocyte pathogenic mechanisms [13,70]. The mutant form of *TRPC6* that causes FSGS also increases intracellular Ca^{2+}. Multiple studies have shown that downstream Ca^{2+} signaling participates in podocyte damage in FSGS after the delayed inactivation of *TRPC6*. Ca^{2+} activates calcineurin [71] and CaMK4 [50], resulting in a decrease in nephrin and synaptopodin expressions, which are critical for podocyte structure. The gain-of-function TRPC6 mutant led to the phosphorylation of ERK1/2 in podocytes, which is involved in the regulation of cell proliferation and survival [72]. However, the pathogenic role of *TRPC6* activation may be through activating NFAT-dependent transcription [73]. As substrates of calcineurin, activation of NFATc transcription factors leads to podocyte dysfunction, and ultimately, to glomerulosclerosis [73].

The loss-of-function *TRPC6* mutation has been shown to be associated with the pathogenesis of FSGS, although the mechanism is currently unclear [67]. Farmer *et al*. [55] reported that *TRPC6*$^{-/-}$ podocytes showed decreased motility, stronger adhesion, and an altered actin cytoskeleton. FAK cleavage is involved in cell death and motility, whereas talin-1 cleavage is associated with cell-adhesion breakdown [74]. Calpain activity was lost and appeared to be mislocalized after the knockdown of *TRPC6*. In addition, cleavage of the calpain targets, talin-1 and FAK, was reduced [55], which may account for the pathogenesis of the *TRPC6* loss-of-function mutation.

In addition to the functional changes of *TRPC6* and downstream pathways contributing to podocyte damage in FSGS, RyR2 may also play a role in the pathogenesis of FSGS. In the NS/FSGS mouse model, the RyR2 channels in the ER of podocytes are phosphorylated, which leads to Ca^{2+} depletion and stress in the ER, resulting in podocyte damage [28].

4.2 Diabetic Kidney Disease (DKD)

An increase in Ca^{2+}-channel activity was observed in the diabetic kidney disease model [14]; the knockdown of Ca^{2+} channels in Akita mice promoted insulin resistance and aggravated glomerular injury [75]. These results highlight the importance of the roles Ca^{2+} channels perform in the pathophysiological state of DKD.

As a metabolic-related kidney disease, the underlying pathogenesis of DKD, especially in the dysfunction of mitochondria, is gradually gaining attention [76]. Several studies have shown that Ca^{2+} signaling participates

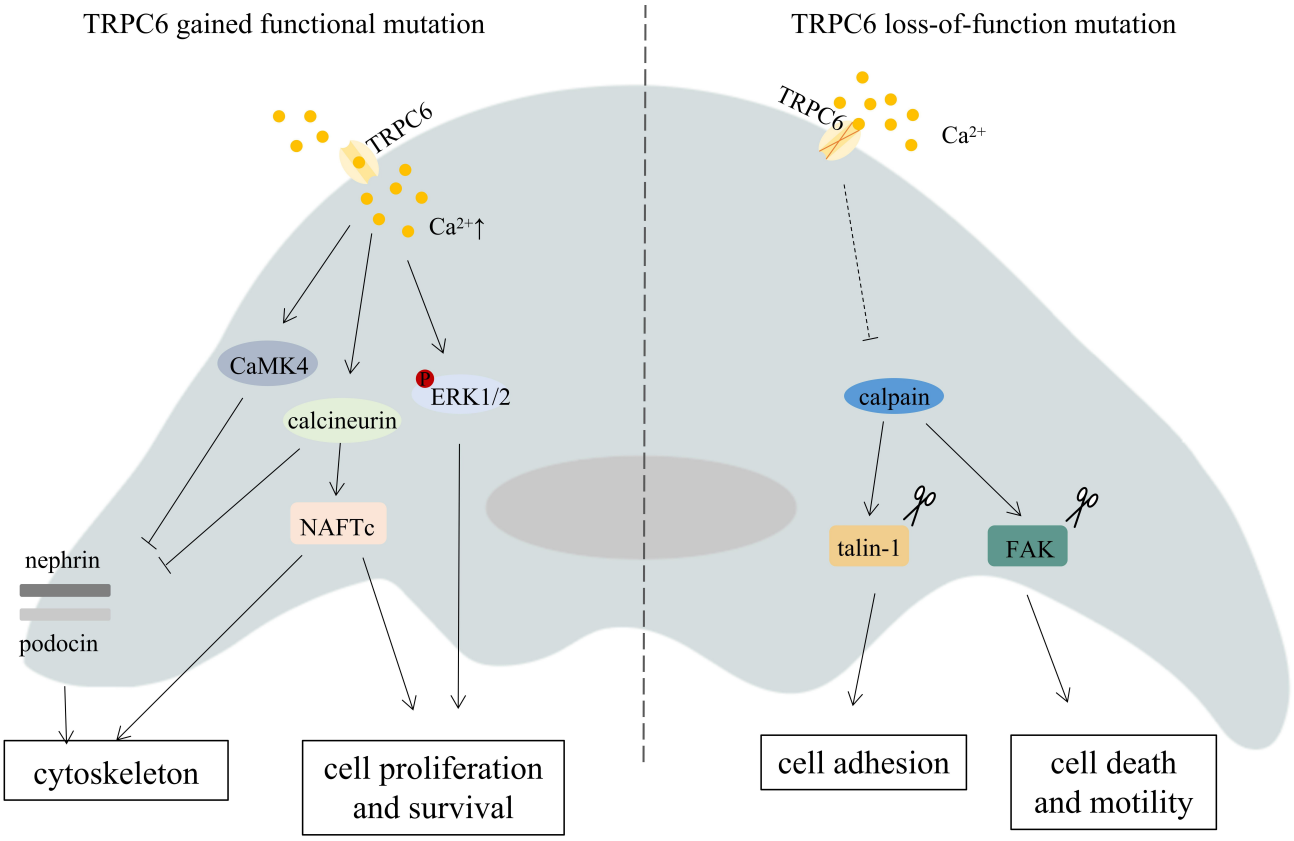

Fig. 1. Calcium signaling in focal segmental glomerulosclerosis.

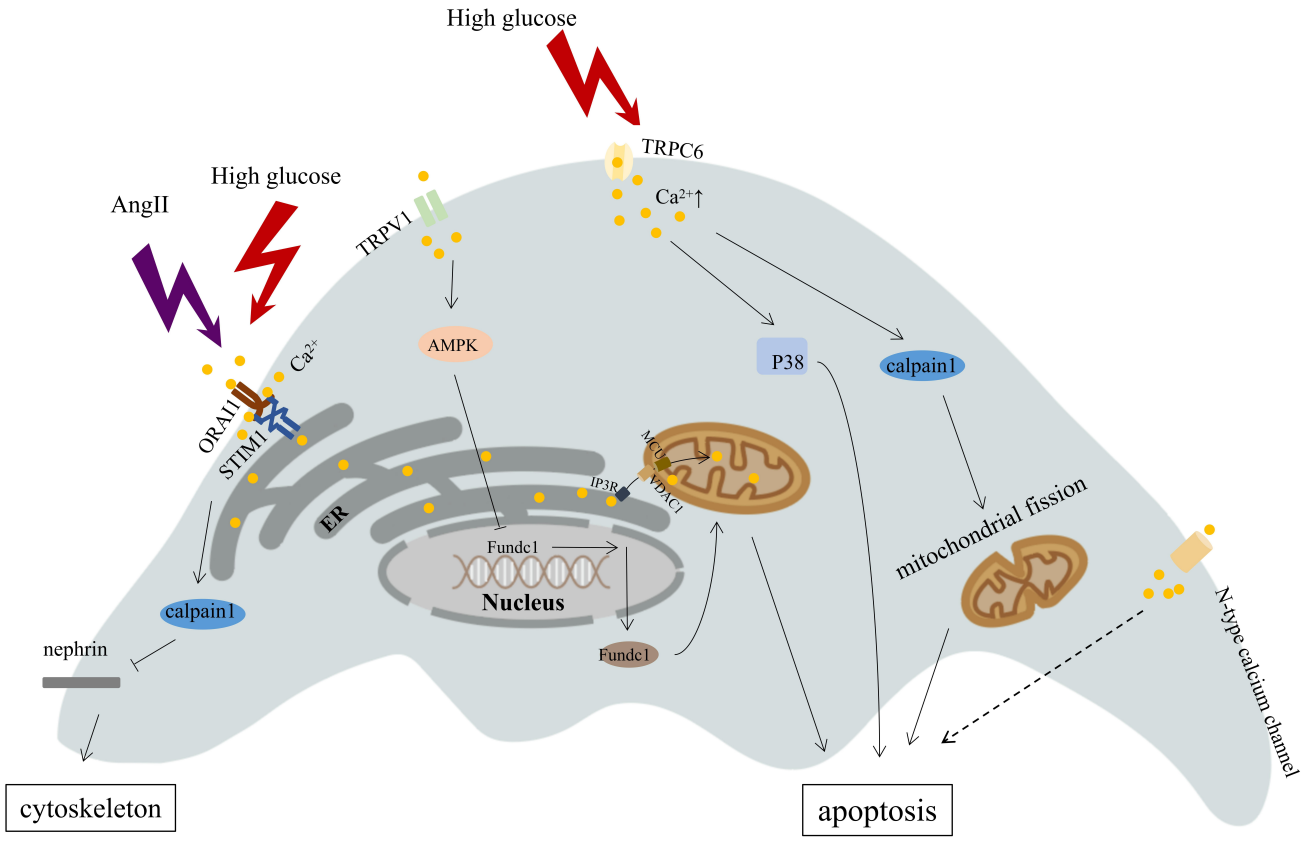

Fig. 2. Calcium signaling in diabetic kidney disease.

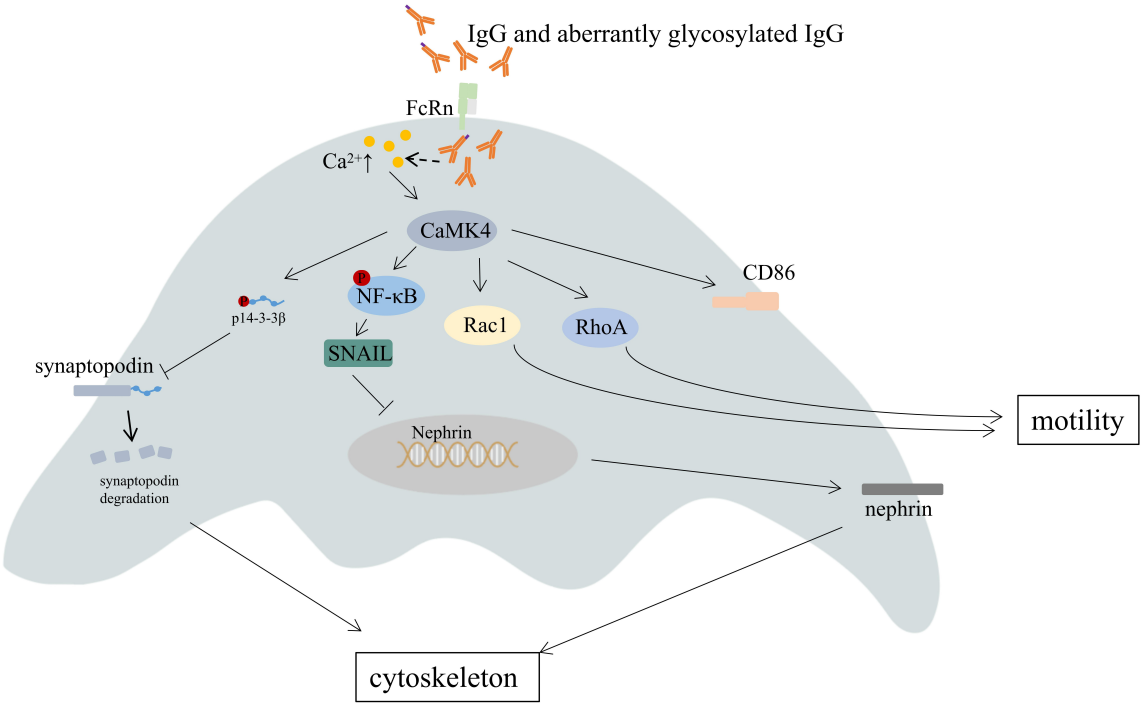

Fig. 3. Calcium signaling in lupus nephritis.

in the development of podocyte injury in DKD by regulating mitochondrial function (Fig. 2). Yu *et al.* [77] reported that TRPC6 mediated high-glucose-induced mitochondrial fission in DKD podocyte injury. TRPC6 mediates mitochondrial fission by Ca^{2+}-activated calpain1 [77]. Tao *et al.* [78] found that an increase in SOCE in podocytes was induced by high glucose and angiotensin II (AngII), thereby mediating impaired mitochondrial membrane potential (MMP), ATP and mitochondrial superoxide production, and mitochondrial respiratory damage in DKD. Disruption of Ca^{2+} homeostasis in mitochondria leads to increased oxidative stress and cell apoptosis [79]. The connection between the "mitochondria-associated membrane" (MAM) and the ER can maintain Ca^{2+} homeostasis in the mitochondria [80]. Wei *et al.* [81] reported that the activation of TRPV1 attenuated mitochondrial dysfunction in podocytes caused by hyperglycemia. Transient Ca^{2+} influx, mediated by TRPV1, reduced the transcription of Fundc1, a key molecule involved in MAM formation, by activating 5′ AMP-activated protein kinase (AMPK). Therefore, the activation of TRPV1 reduced MAM formation and the transport of Ca^{2+} from the ER to the mitochondria [81]. When the ER releases Ca^{2+} through the inositol 1,4,5,-triphosphate receptor (IP3R), Ca^{2+} enters the mitochondrial membrane space via VDAC1 and finally enters the mitochondrial matrix through the mitochondrial Ca^{2+} unidirectional transport protein (MCU) [82]. High glucose and AngII induced enhanced effects of IP3R-Grp75-VDAC1-MCU, which increased Ca^{2+} in the mitochondria as well as increased active caspase-3 [64]. This process participates in podocyte apoptosis in DKD.

High glucose-induced apoptosis is one of the key factors contributing to podocyte injury in DKD [83]. Upregulation of Ca^{2+}/calcineurin (CaN) signaling promotes podocyte apoptosis, which is a key mechanism in podocyte injury in DKD [84]. Podocyte apoptosis may also relate to the increased expression of p38, caused by TRPC6 knockout in Akita mice [75]. Tao *et al.* [85] also showed that a high glucose-induced increase of SOCE led to decreased expression of the nephrin protein by promoting calpain activation. In addition to the above Ca^{2+} channels, the N-type calcium channel is also involved in proteinuria production due to podocyte damage in DKD [86].

4.3 Lupus Nephritis (LN)

LN, as a chronic immune-complex-mediated renal lesion, is mainly characterized by renal glomerular impairment [87]. Abnormal IgG can be transported and deposited in podocytes via the neonatal Fc receptor (FcRn), causing podocytopathies [88]. These recent studies have indicated that the Ca^{2+}/CaMK4 pathway plays an essential role in IgG-mediated podocyte injury (Fig. 3).

The expression of Ca^{2+}/CaMK4 is significantly elevated in podocytes of LN patients and lupus-prone mice [46]. Ichinose *et al.* [88] showed that pathogenic IgG upregulated CaMK4 and altered the expression of some genes associated with podocyte injury, including CD86 costimulation and skeleton-related genes, such as nephrin and synaptopodin. Bhargava *et al.* [46] also reported that CaMK4 upregulation, mediated by aberrantly glycosylated IgG, can phosphorylate NF-κB, which decreases the expression of nephrin by upregulating SNAIL. In addition,

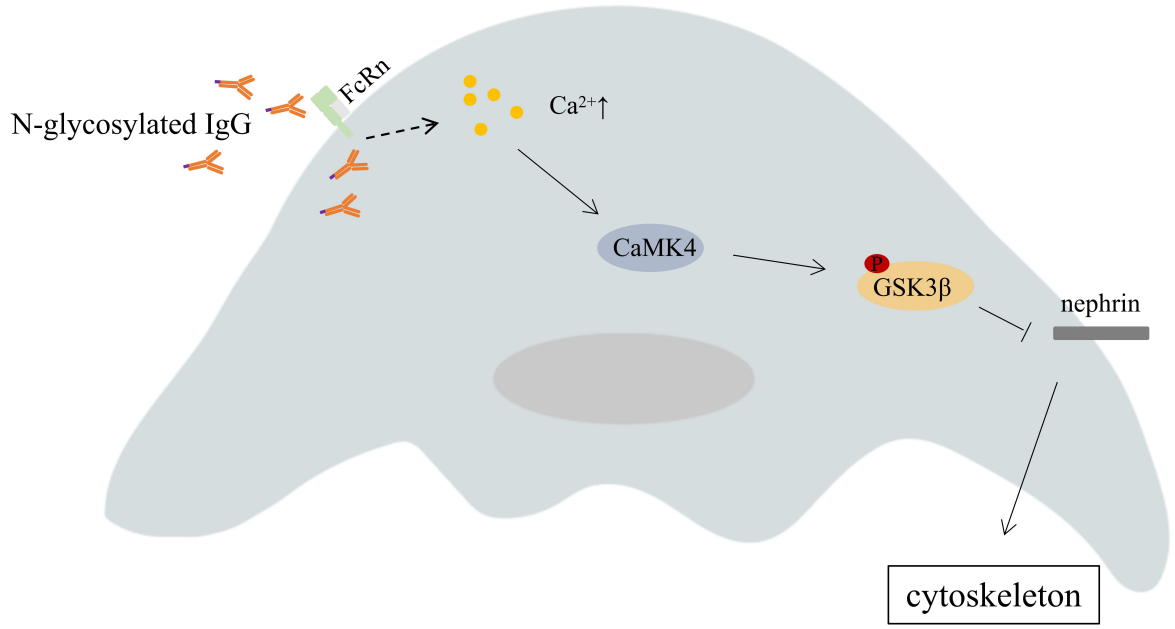

Fig. 4. Calcium signaling in transplant glomerulopathy.

CaMK4 can also modulate podocyte motility in LN through the regulation of Rac1 and RhoA, while can also regulate the degradation of synaptopodin in LN via the phosphorylated scaffold protein 14-3-3β [50]. These studies suggest that Ca^{2+}/CaMK4 may be a valuable target for the treatment of podocyte injury in LN.

4.4 Transplant Glomerulopathy (TG)

Chronic antibody-mediated immune rejection, ultimately, leads to renal graft failure. TG is the main histological feature. Podocyte shedding was closely associated with the occurrence of proteinuria and eGFR decrease in TG [89]. Both TG and LN are thought to be the result of antibody-mediated damage. Bhargava *et al.* [9] reported that N-glycosylated IgG in TG patients can also enter podocytes via FcRn. N-glycosylated IgG activates CaMK4, which phosphorylates GSK3β. Phosphorylated GSK3β reduces nephrin expression, resulting in the regulation of podocyte migration and the rearrangement of the actin cytoskeleton [9] (Fig. 4).

4.5 Hypertensive Renal Injury

Hypertension damages podocytes due to increased pressure in the glomerular capillaries, leading to the loss of podocytes [90]. The activity of RAS is enhanced in hypertensive patients [91]. AngII-induced autophagy in the podocytes of spontaneously hypertensive rats was explored by Ma *et al.* [11]. They found that the activation of TRPC6 can be stimulated by AngII, thereby resulting in Ca^{2+} influx and CaMKKβ-AMPK pathway activation, which leads to podocyte autophagy. N-type calcium channel is also involved in AngII-induced podocyte injury [92], which may be related to the production of ROS caused by AngII [93]. Golosova *et al.* [94] showed that κ-opioid receptors (κORs)/TRPC6 signaling is activated in hypertensive rats and human podocytes, while pathological Ca^{2+} signaling leads to actin cytoskeleton rearrangement. These results suggest a critical role for Ca^{2+} signaling in hypertensive podocyte injury (Fig. 5).

Ca^{2+} channels and Ca^{2+}-signaling pathways are closely related to the pathogenesis and progression of podocyte-related diseases. Activation of Ca^{2+} channels, such as TRPC6, RyR2, SOCs, and VDAC1, has been reported to contribute to podocyte-related diseases. Ca^{2+}/CaMK4 was found to be involved in podocyte injury in LN and TG, and the activation of TRPV1 attenuated mitochondrial dysfunction in podocytes in DKD. These results suggest that Ca^{2+} channels and Ca^{2+} signaling are promising targets for the treatment of podocyte-related diseases.

5. Targets of Ca^{2+}-Signaling Pathways in Podocytes

The implementation of Ca^{2+}-signaling pathways as promising therapeutic targets is the focus of researchers; thus, we summarized the current Ca^{2+}-signaling targets associated with podocytes in Table 2 (Ref. [11,16,28,57,71,86,93,95–110].

5.1 CaSR

The specific knockdown of CaSR in podocytes disrupted the actin cytoskeleton and reduced cell attachment and migration speed, resulting in proteinuria. Calcimimetics allosterically activate the sensitivity of the CaSRs to Ca^{2+}.

Table 2. Drugs and inhibitors targeting Ca^{2+} signaling pathways in podocytes.

Drugs and inhibitors	Targets	Mechanism	Disease	Reference
R-568	CaSR	Activates the sensitivity of CaSR to calcium ions	Glomerulosclerosis	[57]
Cinacalcet			NS	[16]
Atractylodis rhizoma water extract (ARE)	TRPC 6	Inhibits the activation of TRPC 6/p-CaMK4 signaling	Fructose-induced kidney disease	[95,96]
Astragaloside IV (AS-IV)		Inhibits the activation of TRPC 6	DKD	[97]
Tetrandrine		Inhibit TRPC 6 expression and downstream RhoA/Rock and calpain 1 signaling pathway	NS	[98–100]
Taurine (2-aminoethenonic acid)		Suppresses TRPC 6 expression by upregulating H2S synthesis	DKD	[101]
Semi-synthetic compound larixyl carbamate (LC)		Inhibits TRPC6 activity		[102]
Qian Yang Yu Yin (QYYY) granule		Inhibits TRPC6/CaMKKβ pathway	Hypertensive kidney injury	[11]
Metformin		Inhibits TRPC 6 expression through AMPKα1 activation	DKD	[103]
		Inhibits the ZEB 2/TRPC 6 pathway	CKD	[104]
Liraglutide		Reduces TRPC6 expression	DKD	[105]
Sildenafil (Viagra)		Increases cGMP levels by inhibiting PDE 5 and cGMP, to reduce TRPC 6 expression	DKD	[106]
Riociguat		Activates sGC, causing an increase in cGMP production and inhibiting the TRPC 6 pathway	Adriamycin-induced nephropathy	[107]
FK506		Reduces TRPC 6 expression	DKD	[110]
K201	RyR 2	Blocks RyR 2-Ser2808 phosphorylation	NS	[28]
MANF		Restores defective RyR 2 function		
Cilnidipine	Cav 2.2	Inhibits N-calcium channels	DKD	[86,93]
microRNA-30 family members	TRPC, calcineurin, NFATC3	Inhibits TRPC 6, PPP3CA and PPP3CB, PPP3R1 and NFATC3	FSGS	[71]
CNI	calcineurin	Inhibits the calcineurin/NFAT/Angptl4 pathway	NS	[108,109]

Abbreviations: CaSR, calcium-sensing receptor; NS, nephrotic syndrome; DKD, Diabetic Kidney Disease; FK506, tacrolimus; MANF, mesencephalic astrocyte-derived neurotrophic factor; RyR 2, type 2 ryanodine receptor; Cav 2.2, N-type calcium channel; FSGS, Focal Segmental Glomerulosclerosis; TRPC, transient receptor potential canonical; NFATC3, Nuclear factor of activated T cell transcription factors; PPP3CA/B, protein phosphatase 3 catalytic subunit alpha/beta; CNI, calcineurin inhibitors; NFAT, Nuclear factor of activated T cell.

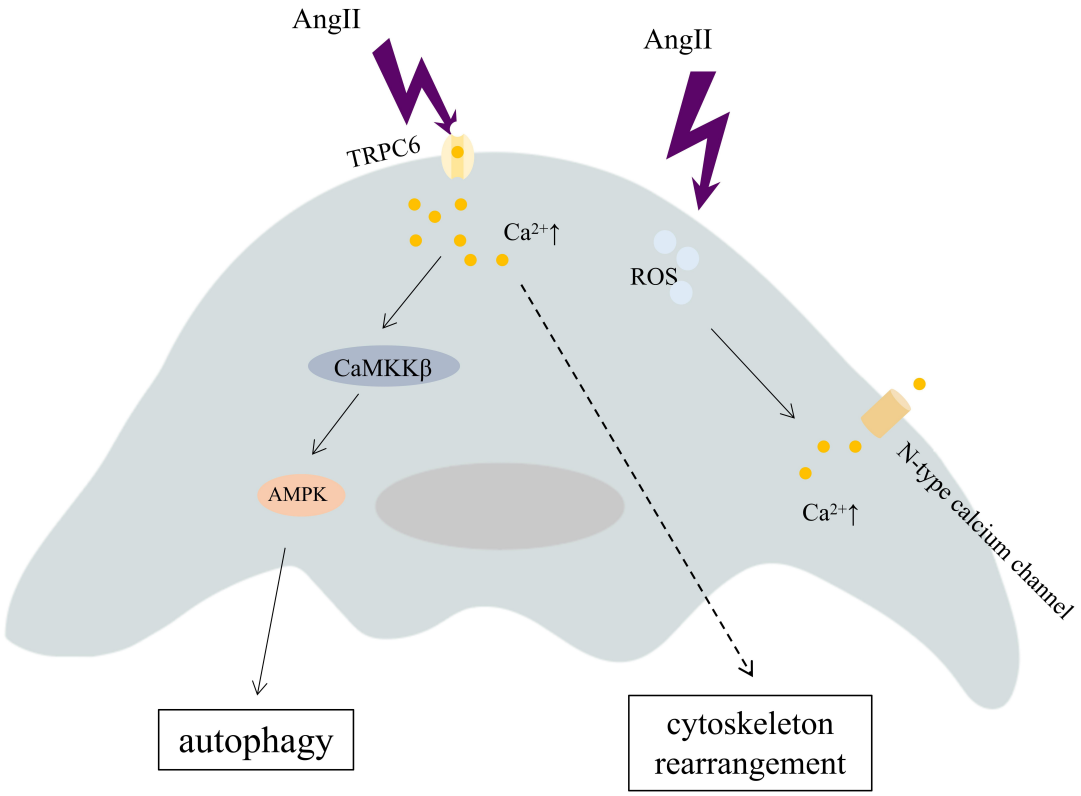

Fig. 5. Calcium signaling in hypertensive renal injury.

Oh *et al*. [57] found that R-568, a calcimimetic that activates the CaSR signal, stabilizes the podocyte cytoskeleton and enhances cell survival, thereby reducing aminonucleoside (PAN)-induced glomerulosclerosis. In addition to R-568, the calcimimetic cinacalcet has also been used in children with nephrotic syndrome to relieve proteinuria [16]. Huang *et al*. [111] proposed that the CaSR has important molecular associations in the pathophysiology of primary membranous nephropathy. Activation of CaSRs may cause podocyte damage through Ca^{2+} imbalance. Yadav *et al*. [112] suggested that the Ca^{2+} imbalance caused by CaSR activation may also be one of the possible mechanisms of renal dysfunction in sepsis. These results suggest that CaSR is also a potential therapeutic target in primary membranous nephropathy and cardio–renal syndrome, which still needs further exploration.

5.2 Ca^{2+} Channels

Therapies targeting Ca^{2+} channels in podocytes are receiving increased attention.

The TRPC6 channel acts as the most important link in podocyte Ca^{2+} signaling. Multiple herbal extracts and synthetics can act to treat podocyte injury by inhibiting the activity of TRPC6 and its downstream pathways. Studies have shown that atractylodin and *Atractylodis rhizoma* water extract (ARE) components can prevent fructose-induced glomerular damage. Atractylodin inhibits the activation of TRPC6/p-CaMK4 signaling in podocyte hypermotility under fructose conditions [95]. ARE also has a therapeutic effect by downregulating the TRPC6/Ca^{2+}/CaMK4 pathway resulting from reducing hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels in the glomeruli [96]. As *Astragaloside IV* (*AS-IV*), an active ingredient isolated from *Astragalus membranaceous* (*Fisch*). Bge, inhibited Ca^{2+} channel activity by targeting TRPC6. *AS-IV* can reduce the Ca^{2+} influx caused by palmitic acid, thereby alleviating the apoptosis of podocytes [97]. Tetrandrine, the main active ingredient isolated from the Chinese herbal radix *Stephania tetrandra*, has been used in a Chinese traditional medicine preparation called *Fangji Huangqi Tang* for the treatment of NS [98]. Studies have indicated that tetrandine can protect podocytes from intracellular Ca^{2+} influx by inhibiting TRPC6 overexpression and downstream RhoA/Rock signaling [99], or the calpain 1 signaling pathway [100]. Taurine (2-aminoethenonic acid) is a semi-essential amino acid, synthesized from cysteine, which inhibits high glucose-induced podocyte damage [101]. Taurine increases cystathionine-γ-lyase (CSE) expression and suppresses TRPC6 expression by upregulating H2S synthesis, thereby alleviating the pathogenic effects of high glucose. Researchers composed a semi-synthetic compound larixyl carbamate (LC), extracted from larixol, as an inhibitor of TRPC 6 channel activity [102]. In addition to synthetic chemicals, *Qian Yang Yu Yin* (QYYY) granule, which is widely used in hypertensive kidney injury, has been shown to protect podocytes by inhibiting the

TRPC6/CaMKKβ pathway [11]. The inhibitory effect of metformin on the TRPC6 pathway has also been demonstrated. Szrejder *et al*. [103] showed that metformin inhibited high-glucose-induced TRPC6 expression in podocytes by activating AMPKα1, thereby protecting the podocyte cytoskeleton. Metformin also abrogates hypoxia-induced podocyte injury by inhibiting the ZEB2/TRPC6 pathway [104]. Liraglutide, a GLP1 receptor agonist, reduced TRPC6 expression in the kidneys of Type 1 diabetic rats, thereby preventing the progression of diabetic kidney disease [105]. Previous drugs used in non-renal diseases were also revived by researchers. Sonneveld *et al*. [106] found that sildenafil (Viagra) increased cyclic guanosine phosphate (cGMP) levels by inhibiting phosphodiesterase type 5 (PDE5)- and cGMP-reduced TRPC6 expression and activity, to prevent the progression of glomerular damage. Hart *et al*. [107] also found that riociguat, through activating soluble guanylate cyclase (sGC), led to an increase in cGMP production, thereby inhibiting the TRPC6 pathway and podocyte damage caused by TRPC6-mediated Ca^{2+} influx. Wang *et al*. [113] reported that an α1-AR agonist (phenylephrine hydrochloride) could participate in the loss of the cytoskeletal structure by inducing a TRPC6-dependent increase in intracellular Ca^{2+} in human podocytes. This provides a possible rationale for the inhibition of TRPC6 by the α1-AR inhibitor (silodosin) [114], to improve podocyte injury.

The Ca^{2+}-release channel RyR2 on the ER of the podocyte is phosphorylated in the NS/FSGS mouse model, causing an imbalance in intracellular Ca^{2+} homeostasis and ER stress. Park *et al*. [28] proposed that a novel compound, K201, blocked RyR2-Ser2808 phosphorylation and that a brain astrocyte-derived neurotrophic factor (MANF) restored the defective RyR2 function.

Albuminuria and ultrafiltration were significantly improved after specific blockade of the α-1 subunit of the N-type calcium channel Ca_v 2.2 in diabetic mice [86]. This suggests that N-type calcium channels are a future therapeutic target for podocyte injury. *Cilnidipine* is an L/N-type dihydropyridine calcium channel blocker (CCB), which can pharmacologically inhibit the N- type calcium channels [115]. Cilnidipine reduced the urinary protein/creatinine ratio in hypertensive patients with chronic kidney disease more effectively than *amlodipine* (L-type CCB) [116,117]. A subsequent study suggested that it may reduce the AngII-induced damage through the inhibition of N-type calcium channels in podocytes [93].

5.3 Ca^{2+}/Calcineurin Signaling

Presently, studies targeting Ca^{2+}/calcineurin are of great interest to researchers. Wu *et al*. [71] proposed that microRNA-30 family members could inhibit the PAN-induced increase in Ca^{2+}/calcineurin signaling in podocytes by targeting the inhibition of TRPC6 (Ca^{2+} channel), PPP3CA and PPP3CB (calcineurin α-catalytic subunit members [118]), PPP3R1 (calcineurin β-regulatory subunit member [118]), and NFATC3 (transcription factor). The antiproteinuric effect and podocyte protection of calcineurin inhibitors (CNI) have been confirmed [108]. Cyclosporine (CsA) and tacrolimus (FK506) are currently the most widely used calcineurin inhibitors [119]. CNI can ameliorate PAN-induced podocyte damage and apoptosis by inhibiting the CaN/NFAT/Angptl4 pathway [109]. Calcineurin can dephosphorylate synaptopodin, be degraded by CatL, and alter the morphology of the podocyte cytoskeleton. CNI destabilizes the podocyte cytoskeleton by targeting calcineurin [120]. In addition, one study showed that FK506 could reduce TRPC6 expression of the podocyte in DKD [110]. The specific mechanism for the beneficial effect of CNI on podocyte injury requires further exploration.

6. Conclusions

Ca^{2+} in podocytes can be stimulated by different signals and transmitted to multiple downstream signaling pathways. Podocytes sense intracellular Ca^{2+} concentrations through the CaSR, generating responses that regulate the balance of Ca^{2+}, which is essential for the function of the glomerular filtration barrier. Similarly, the downstream signaling pathways mediated by the activity of Ca^{2+} channels are involved in various biological functions, such as the morphogenesis, structure, and survival of podocytes. In addition to the TRPCs widely studied in podocytes, recent studies have also suggested the importance of N-type calcium channels for Ca^{2+} transport in podocytes. RyR2, VDAC1, and SOCs are also important for maintaining organelle Ca^{2+} homeostasis. Increased Ca^{2+} influx and Ca^{2+} signaling activation in various podocyte-related diseases can achieve cell communications. FSGS, as one of the diseases most commonly manifested as SRNS, is associated with mutations in various Ca^{2+}-signaling-pathway-related genes. Therapies targeting Ca^{2+} signaling have broad application prospects in patients with FSGS and also provide a novel option for therapy in patients with SRNS. Podocytopathy in DKD is widely recognized, and some progress has been made in the study of Ca^{2+} signaling in podocyte damage that has been induced by high levels of glucose and AngII. However, the effect of focusing Ca^{2+}-signaling therapy on podocyte damage in DKD still needs further exploration. Abnormal IgG-antibody-mediated podocyte injury in LN and TG also gradually attracted the interest of researchers to the role of Ca^{2+}; however, it too requires additional exploration. Ca^{2+} signaling targets may also be used as treatment strategies for immune-related kidney injuries. Currently, the therapeutic strategies targeting the Ca^{2+}-signaling pathway focus on channel proteins, yet with the research progress on CaSRs and downstream signaling of Ca^{2+}, they will soon be researched as potential therapeutic targets.

Author Contributions

YCT and LJY selected the topic. YCT wrote the paper. LJY provided guidance to the paper. LJY, YCT, HPS, LLS, QQL, LF, and MR participated in the discussion, revision, and correction of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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