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The Finer Points of Podocyte Sphingolipid Metabolism in Diabetic Kidney Disease

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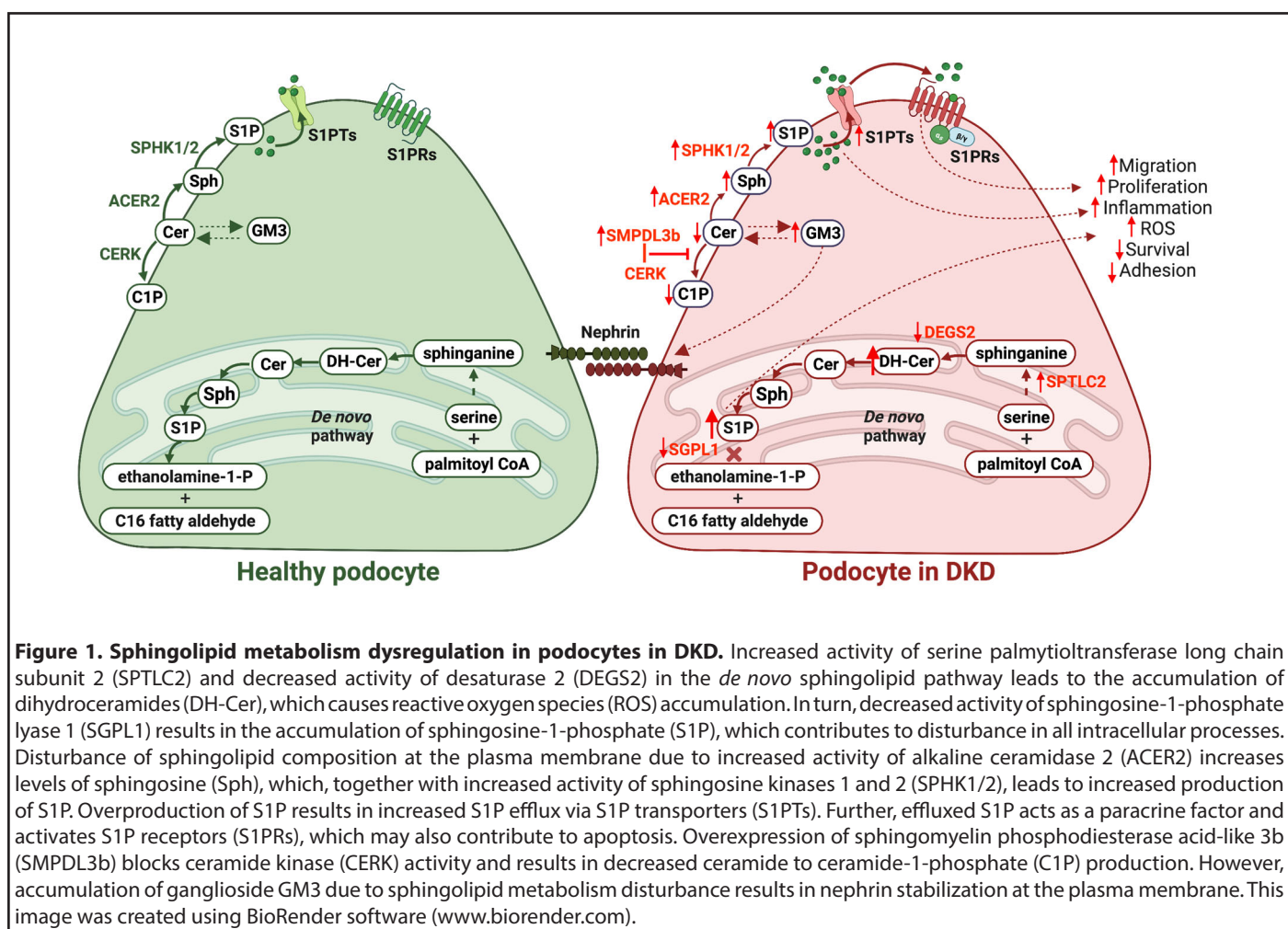
Commentary

Diabetic kidney disease (DKD) is a common complication of diabetes [1], characterized by kidney damage. Podocytes are specialized, terminally differentiated cells in the kidney's filtration barrier that are key responders to the metabolic and environmental changes that occur in diabetes. Change in the function and in the number of podocytes is the main signature of the development and progression of DKD. However, the exact causes of podocyte injury and detachment in DKD are not fully understood. Recent research has shed light on the intricate role that sphingolipid metabolism may play in podocyte injury and its relevance to DKD progression, as discussed in this commentary.

Sphingolipids belong to a large class of lipid molecules which are involved in various cellular processes, including cell membrane structure, cell signaling and proliferation, inflammation, oxidative stress, or apoptosis. The most critical role sphingolipids play is in the lipid raft domains, the sphingomyelin-, cholesterol- and glycosyl-phosphatidylinositol (GPI)-rich microdomains of the plasma membrane. The slit diaphragm of podocytes is a specialized structure that resembles a lipid raft and organized into a complex network of transmembrane proteins such as nephrin, podocin, alpha-actinin-4, signaling adaptors CD2AP and ion channels like TRPC6. The proper localization and function of slit diaphragm proteins depend largely on bioactive sphingolipids. Changes in the sphingolipid composition of lipid raft domains affect the biophysical properties of the cell plasma membrane, leading to changes in signaling properties and further podocyte injury (Reviewed in [2]). Ceramide, ceramide-1-phosphate (C1P), sphingosine-1-phosphate (S1P), glycosphingolipids (particularly, gangliosides) and galactosylceramides (GalCer) are the main sphingolipid metabolites that have been considered as bioactive signaling sphingolipids in podocytes (**Figure 1**).

It is important to note that hyperglycemia is the key determinant of podocyte injury in diabetes. Therefore, high glucose levels significantly contribute to disturbance in sphingolipid metabolism. Thus, excessive glucose has been shown to increase glycosphingolipid production by elevating the availability of ceramide [3]. Another study demonstrated that elevated levels of advanced glycation end-products lead to increased levels of gangliosides in renal cells [4]. Ceramides have also been implicated in insulin resistance via interfering with glucose uptake and impaired storage of glycogen or triglycerides (Reviewed in [5]). Increased production of ceramides is linked to elevated activity of serine palmitoyltransferase, the *de novo* ceramide synthesis pathway enzyme, in the presence of high glucose levels [6], while decreased activity of acid ceramidase, an enzyme responsible for degradation of ceramide, has been also reported in the hyperglycemia conditions in association with increased inflammatory response [7].

The knowledge about podocyte sphingolipid metabolism under physiological and pathophysiological conditions is scarce. Recent experimental studies however suggest that

Figure 1. Sphingolipid metabolism dysregulation in podocytes in DKD. Increased activity of serine palmytioltransferase long chain subunit 2 (SPTLC2) and decreased activity of desaturase 2 (DEGS2) in the *de novo* sphingolipid pathway leads to the accumulation of dihydroceramides (DH-Cer), which causes reactive oxygen species (ROS) accumulation. In turn, decreased activity of sphingosine-1-phosphate lyase 1 (SGPL1) results in the accumulation of sphingosine-1-phosphate (S1P), which contributes to disturbance in all intracellular processes. Disturbance of sphingolipid composition at the plasma membrane due to increased activity of alkaline ceramidase 2 (ACER2) increases levels of sphingosine (Sph), which, together with increased activity of sphingosine kinases 1 and 2 (SPHK1/2), leads to increased production of S1P. Overproduction of S1P results in increased S1P efflux via S1P transporters (S1PTs). Further, effluxed S1P acts as a paracrine factor and activates S1P receptors (S1PRs), which may also contribute to apoptosis. Overexpression of sphingomyelin phosphodiesterase acid-like 3b (SMPDL3b) blocks ceramide kinase (CERK) activity and results in decreased ceramide to ceramide-1-phosphate (C1P) production. However, accumulation of ganglioside GM3 due to sphingolipid metabolism disturbance results in nephrin stabilization at the plasma membrane. This image was created using BioRender software (www.biorender.com).

decreased levels of long- (C14:0, C16:0, C18:0) and very-long- (C24:0, C24:1) chain ceramide species in mice kidney cortices are associated with altered genes expression involved in ceramide synthesis and metabolism [8-10], while human studies suggest an inverse correlation between ceramide long- (C16:0) and very-long- (C24:0) chain species in plasma and kidney cortex [11] and elevated urinary ceramide species [12]. Ceramide accumulation in glomeruli mice with DKD is also associated with expression of junctional adhesion molecule-like protein (JAML) [13]. Importantly, not only ceramide synthesized via *de novo* pathway are toxic to podocytes, but ceramides produced by the action of ceramidases or sphingomyelinases have also been shown to be equally toxic. Thus, podocyte-specific acid ceramidase 1 (*Asah1*) deletion in mice leads to increased glomerular ceramide levels and development of nephrotic syndrome [14], which is ameliorated by the deletion of acid sphingomyelinase (*Smpd1*) gene [15]. *Asah1* has also been shown to play an important role in podocyte injury by promoting nicotine adenine dinucleotide phosphate (NADPH) oxidase associated oxidative stress [16], suggesting an existence of a crosstalk between sphingolipids and NADPH oxidase signaling (as reviewed in [17]). However, many aspects of the role of ceramide metabolism in the podocyte remain

to be discovered. One of the most important questions to be answered is the role of different ceramide species in DKD development, as short-chain ceramides are generally associated with pro-survival and anti-inflammatory effects, while long-chain ceramides are often linked to inflammation and apoptosis.

Ceramide can be further catabolized into sphingosine, which is then phosphorylated into S1P, a bioactive sphingolipid that has gained a lot of attention in recent years. Very often, S1P and ceramide antagonistically signal cell survival or death largely via shared mediators, and both can directly inhibit each other's synthesis. S1P primarily acts as a ligand for high-affinity five cell-surface receptors (S1Pr1-S1Pr5). However, its intracellular actions may also possess signaling potential, despite the fact that its direct targets remain elusive and controversial [18]. Loss of S1P lyase, an enzyme that degrades S1P, has been shown to be associated with nephrotic syndrome [19,20]. In podocytes, this loss resulted in a reduction of nephrin, an important protein of a slit diaphragm, via a protein kinase C δ-dependent mechanism [21]. Additionally, the levels of sphingosine kinase, an enzyme that catalyzes S1P formation, are increased in DKD, leading to increased renal S1P levels as

shown in rodent models [22,23] and in patients with DKD [9,22]. Abnormalities in S1P receptors expression also contribute to DKD progression (**Figure 1**), where S1P signaling mediated by S1Pr2 is shown to be toxic to cells, while S1Pr1 signaling has a protective role [24]. While significant progress has been made in understanding S1P signaling in podocytes, several questions remain unanswered. The relative contribution of intracellular and extracellular S1P remains to be established. Furthermore, the relative expression and activation of S1P receptors in podocytes during DKD, and the downstream signaling pathways involved, remain largely unexplored. Finally, it also remains unclear what the intracellular resources of S1P are in the podocyte, as well as how S1P transport and distribution occurs within the cell. Elucidating S1P crosstalk with other signaling pathways, such as renin-angiotensin system, oxidative stress, or inflammation-related pathways, needs further investigation and may lead to the development of targeted therapeutic intervention.

Ceramide can also be phosphorylated into C1P by ceramide kinase (CERK), another bioactive sphingolipid involved in diverse intracellular processes including survival, proliferation, migration through an unknown receptor-initiated signaling or inflammation. The function of C1P in the podocyte remains largely unknown. We recently demonstrated decreased levels of C1P in kidney cortices of mice with DKD [8]. This was associated with increased expression of sphingomyelin phosphodiesterase acid-like 3b (SMPDL3b), an enzyme of the sphingolipid pathway that modulates insulin receptor signaling and that regulates C1P availability in podocytes via interfering with CERK expression [25]. Given the fact that C1P is heavily involved in the cellular processes, as shown in other cell types, its role in podocytes remains largely unknown and requires further investigation.

In addition, the role of glucosylceramides (glucosylcerebrosides, lactocylceramides and gangliosides) in podocyte biology and in DKD has recently gained some attention. Thus, glucosylceramide C18:0 is increased in plasma of mouse models of DKD, while glucosylceramide C16:0 is decreased in the DKD kidney [10]. In previous studies, ganglioside GM3, the most abundant sphingolipid in podocytes, has been found to be up-regulated in DKD and implicated in insulin resistance [26], while a recent study demonstrated that increased GM3 levels stabilize nephrin and prevent podocyte loss and albuminuria [27]. Increased levels of hexocyl-, glucosyl-, galactosyl- and lactosylceramides were observed in kidney cortices of mouse model of DKD [3]. Another study reported an association between hexosylceramide C18:1 and DKD, while very-long-chain lactosylceramide species are associated with microalbuminuria development in patients with type 1 diabetes [28].

Understanding the intra- or extracellular factors that drive sphingolipid pathophysiology in podocytes, as well as the contribution of specific sphingolipid species to podocyte physiology and pathophysiology is likely to result in the development of novel diagnostic biomarkers and novel therapeutic opportunities. Numerous studies have investigated the potential of sphingolipids as diagnostic biomarkers for DKD. These studies have identified specific changes in the levels of sphingolipids in the blood or urine of DKD patients compared to individuals without DKD or with other types of kidney disease. For example, recent research has established links between urinary ceramides and the various stages of diabetic nephropathy. In this study, certain ceramides such as Cer d18:1/16:0, Cer d18:1/18:0, Cer d18:1/20:0, Cer d18:1/22:0, and Cer d18:1/24:0 were found to be elevated in patients with stage 3 of diabetic nephropathy. Furthermore, the levels of these ceramides in urine were positively associated with urinary clinical biomarkers such as albumin and N-acetyl-β-d-glucosaminidase, as well as the sediment score [12]. In addition, a clinical study demonstrated increased levels of C16 and C18 ceramides in the plasma of patients with type 2 diabetes (T2D) [29]. Similarly, elevated levels of C16 and C18 ceramides were found in the urine of DKD patients, and these levels were correlated with urinary biomarkers such as albumin [10]. Conversely, another study revealed that lower plasma levels of very long chain ceramide species (C20–C26) were associated with the progression of proteinuria in DKD [30]. These findings indicate that sphingolipids, particularly ceramides, may not only serve as diagnostic biomarkers for DKD but also as indicators of disease progression and prognosis. In addition to their potential as diagnostic biomarkers, the identification of sphingolipids as key players in DKD has led to the exploration of sphingolipid-targeted therapeutic interventions. Activation of sphingosine-1-phosphate receptor 1 (S1P1R) has been shown to have protective effects on renal function and histology in an STZ-induced diabetic rat model [31]. Furthermore, we have previously demonstrated that supplementation with exogenous C1P may represent a lipid therapeutic strategy to treat DKD [8]. However, further research is needed to better understand the complex sphingolipid signaling network and validate the efficacy and safety of sphingolipid-targeted therapies in clinical settings.

Therefore, our rudimentary understanding of the role of sphingolipid metabolism in podocytes in health and disease is expanding and paves the way for further investigations.

Disclosure

Mitrofanova A, Njeim R, Fornoni A. The Finer Points of Podocyte Sphingolipid Metabolism in Diabetic Kidney Disease. J Cell Signal. 2023;4(3):99-103.

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