Semaphorins and Plexins in Kidney Disease

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**Key Words**
Semaphorin · Plexin · Diabetic nephropathy · Glomerulosclerosis · Glomerulonephritis · Injury · Repair · Systemic lupus erythematosus · Lupus nephritis · Hypertension · Podocyte · Tubule · Immune · Inflammation · Fibrosis · Mitotic spindle · Cell division

**Abstract**
Semaphorins are soluble or membrane-bound cues, which control multiple aspects of cell-cell communication, differentiation, morphology and function. Most of their effects are mediated by a family of transmembrane receptors called plexins. Semaphorins and plexins have emerged as central regulators of diverse physiological and pathophysiological processes in various organs. This review summarizes the role of semaphorins and plexins in renal pathophysiology and their potential use as biomarkers of kidney disease.

**Introduction**
Semaphorins comprise a family of membrane-bound or diffusible factors that were initially discovered for their role as axon guidance cues in the developing nervous system. They are now recognized as key regulators of multiple cellular functions in the renal, immune, nervous, bone and cardiovascular systems, both during development as well as in the adult organism [1, 2]. In mammals, 20 semaphorins have been identified, which are grouped into 5 classes, 3–7, based on structural characteristics (fig. 1). Plexins, a family of single-pass transmembrane proteins, represent the main semaphorin receptors and are classified into 4 subfamilies, A–D (fig. 1). The structural hallmark of semaphorins and plexins is the Sema domain, a ~500-amino-acid seven-blade \( \beta \)-propeller fold, which mediates the semaphorin–plexin interaction. In some cases, the binding of semaphorins to plexins is stabilized by plexin co-receptors, the neuropilins [1] (fig. 1). Multiple semaphorins and plexins are expressed during kidney organogenesis and have been shown to control diverse developmental processes like branching of the ureteric bud, establishment of the glomerular filtration barrier and patterning of the renal vasculature [3, 4]. In the adult, semaphorin–plexin signaling is crucially involved in multiple aspects of renal pathophysiology. This review focuses on the biological roles of semaphorins and plexins and their potential as biomarkers in kidney disease.
Semaphorins and Plexins in Glomerular Diseases

Semaphorin 3A in Diabetic Nephropathy

Diabetes mellitus is the leading cause of chronic kidney disease and affects primarily the renal corpuscle. The clinical manifestations of diabetic nephropathy, proteinuria and a progressive loss of glomerular function, correlate with histological changes that are characterized by thickening of the glomerular basement membrane and glomerular sclerosis. Sema3A, a soluble, secreted semaphorin (fig. 1), has been found to be expressed in immortalized murine podocytes in vitro as well as in podocytes of adult humans, mice and rats in vivo [5–8] (table 1). In a mouse model of diabetes, and in diabetic patients, Sema3A expression levels in podocytes are elevated [8, 9]. Evidence for a functional significance of Sema3A in kidney physiology and diabetic nephropathy comes from gain-of-function and loss-of-function studies in mice us-

**Fig. 1.** The mammalian semaphorin–plexin system. In mammals, 20 semaphorins (grouped in 5 classes, 3–7) and 9 plexins (classified in 4 subfamilies, A–D) have been identified so far, all of which share the Sema domain as their common feature. While class-3-semaphorins are secreted, semaphorins of other classes are membrane bound. Some class-4-semaphorins can be released from the cell membrane by proteolytic cleavage. The intracellular portion of all plexins comprises a GAP domain, which is separated into 2 parts by a RBD. In some cases, the semaphorin–plexin interaction requires stabilization by neuropilin-1 or -2. PSI = Plexin-semaphorin-integrin; Ig = immunoglobulin; PDZ = post synaptic density 95/discs large/zonula occludens-1; GPI = glyкопosphatidylinositol; IPT = immunoglobulin-plexin-transcription factor; RBD = Rho GTPase-binding domain; SEA motif = consists of the amino acids serine, glutamic acid and alanine; MAM = meprin-A-5 protein-receptor protein tyrosine phosphatase mu.
### Table 1. Expression of semaphorins and plexins in the adult kidney

<table>
<thead>
<tr>
<th>Expression in normal kidney</th>
<th>Species</th>
<th>Expression changes in disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Semaphorins</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sema3A Podocytes</td>
<td>Human</td>
<td>↑ in diabetic nephropathy</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>↑ in STZ-induced diabetes</td>
<td>[5–7, 9]</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>↓ in diabetic nephropathy</td>
<td>[7]</td>
</tr>
<tr>
<td>TECs of distal tubules and</td>
<td>Human</td>
<td>↔ in diabetic nephropathy</td>
<td>[8]</td>
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<tr>
<td>collecting ducts</td>
<td>Mouse</td>
<td>↑ and additional expression in TECs of proximal tubules in ischemia/reperfusion injury</td>
<td>[7, 9, 17, 18]</td>
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<td></td>
<td>Rat</td>
<td>↑ in cisplatin-induced acute kidney injury</td>
<td>[7, 9, 17, 18]</td>
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<tr>
<td></td>
<td></td>
<td>↑ in STZ-induced diabetes</td>
<td>[7, 9, 17, 18]</td>
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<tr>
<td>Sema3C Podocytes (immortalized)</td>
<td>Mouse</td>
<td>↑ in ischemia/reperfusion injury</td>
<td>[13]</td>
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<tr>
<td>Sema3D Podocytes (immortalized)</td>
<td>Mouse</td>
<td>↔ in STZ-induced diabetes</td>
<td>[6, 9, 18]</td>
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<tr>
<td>Sema3E Podocytes (immortalized)</td>
<td>Mouse</td>
<td>↔ in ischemia/reperfusion injury</td>
<td>[13, 21]</td>
</tr>
<tr>
<td>Sema3F Podocytes</td>
<td>Mouse, rat</td>
<td>↓ in ischemia/reperfusion injury</td>
<td>[6, 9, 18]</td>
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<tr>
<td>TECs of distal tubules and</td>
<td>Mouse, rat</td>
<td>↑ in ischemia/reperfusion injury</td>
<td>[6, 9, 18]</td>
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<tr>
<td>collecting ducts</td>
<td></td>
<td>↔ in STZ-induced diabetes</td>
<td>[6, 9, 18]</td>
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<td>Sema4A TECs of proximal tubes</td>
<td>Mouse</td>
<td>↓ in ischemia/reperfusion injury</td>
<td>[13]</td>
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<tr>
<td>Sema4B TECs of all tubular segments</td>
<td>Mouse</td>
<td>↑ in ischemia/reperfusion injury</td>
<td>[13]</td>
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<tr>
<td>Sema4C Glomeruli</td>
<td>Mouse</td>
<td>↔ in ischemia/reperfusion injury</td>
<td>[13, 21]</td>
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<td>Interstitial cells</td>
<td>Mouse</td>
<td>↑ in ischemia/reperfusion injury</td>
<td>[13, 21]</td>
</tr>
<tr>
<td>Sema4D TECs of proximal tubules</td>
<td>Mouse</td>
<td>↔ in ischemia/reperfusion injury</td>
<td>[12–14]</td>
</tr>
<tr>
<td>Sema4F Whole kidney (localization unclear); expressed at low levels</td>
<td>Mouse</td>
<td>↔ in ischemia/reperfusion injury</td>
<td>[13]</td>
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<tr>
<td>Sema4G Whole kidney (localization unclear); expressed at high levels</td>
<td>Mouse</td>
<td>↑ or ↓ in ischemia/reperfusion injury (depending on time point after injury)</td>
<td>[13]</td>
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<td><strong>Plexins</strong></td>
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<tr>
<td>Plexin-A1 Podocytes (immortalized)</td>
<td>Mouse</td>
<td>↓ in STZ-induced diabetes</td>
<td>[6, 9, 18]</td>
</tr>
<tr>
<td>Whole kidney (localization uncertain)</td>
<td>Mouse</td>
<td>↓ in ischemia/reperfusion injury</td>
<td>[6, 9, 18]</td>
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<tr>
<td>Plexin-A2 Podocytes (immortalized)</td>
<td>Mouse</td>
<td>↓ in STZ-induced diabetes</td>
<td>[9, 18]</td>
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<tr>
<td>Whole kidney (localization uncertain)</td>
<td>Mouse</td>
<td>↓ in ischemia/reperfusion injury</td>
<td>[9, 18]</td>
</tr>
<tr>
<td>Plexin-A3 Podocytes (immortalized)</td>
<td>Mouse</td>
<td>↓ in STZ-induced diabetes</td>
<td>[9, 18]</td>
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<tr>
<td>Whole kidney (localization uncertain)</td>
<td>Mouse</td>
<td>↓ in ischemia/reperfusion injury</td>
<td>[9, 18]</td>
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<td>Plexin-B1 Glomeruli</td>
<td>Mouse</td>
<td>↑ in STZ-induced diabetes</td>
<td>[12, 21]</td>
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<tr>
<td>TECs of distal tubules and</td>
<td>Mouse</td>
<td>↑ in STZ-induced diabetes</td>
<td>[14]</td>
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<tr>
<td>collecting ducts</td>
<td></td>
<td>↓ in ischemia/reperfusion injury</td>
<td>[14]</td>
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<tr>
<td>Glomeruli</td>
<td>Mouse</td>
<td>↑ in STZ-induced diabetes</td>
<td>[21]</td>
</tr>
<tr>
<td>TECs of all tubular segments</td>
<td>Mouse</td>
<td>↑ in ischemia/reperfusion injury</td>
<td>[13, 21]</td>
</tr>
<tr>
<td>Plexin-D1 Podocytes (immortalized)</td>
<td>Mouse</td>
<td>↓ in ischemia/reperfusion injury</td>
<td>[5]</td>
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STZ = Streptozotocin; TEC = tubular epithelial cell; n.a. = not assessed.
ing both pharmacological as well as genetic approaches (fig. 2a). Systemic administration of exogenous recombina
t Sema3A into mice or doxycycline-inducible, podocy-
cystic-specific overexpression of Sema3A in transgenic
mice induce marked morphological abnormalities in the
renal corpuscle including podocyte damage and glomer-
ular endothelial swelling, which are accompanied by pro-
teinuria [6, 10]. Consistent with these effects of Sema3A
in normal mice, podocyte-specific overexpression of Sema3A in diabetic mice worsens glomerulosclerosis
and kidney function [8]. Conversely, diabetic mice expressing
mutant Sema3A, and diabetic mice injected with a pep-
tide that blocks Sema3A-induced effects in neurons [11]
show less albuminuria, attenuated glomerulosclerosis
and interstitial fibrosis, and a decrease in macrophage in-
filtration and in the renal expression of pro-inflammatory
and pro-fibrotic genes, while kidney function is im-
proved [9]. Interestingly, treatment of diabetic mice with
a Sema3A-inhibitory peptide is effective after the onset of
proteinuria, suggesting that approaches targeting Sema3A
might hold therapeutic potential [9]. Mechanistically, ex-
cessive Sema3A impacts on the integrity of the slit dia-
aphragm by interfering with the nephrin-podocin-CD2AP
complex (fig. 2a). Nephrin is a transmembrane adhesion molecule
that forms homophilic interactions between neighboring

![Fig. 2. Semaphorins and plexins in glomerular diseases. a Sema3A is expressed by podocytes (dashed arrow) and binds to its receptor Plexin-A1 on podocytes, which interacts with nephrin (left). In diabetic nephropathy, Sema3A expression in podocytes is upregulated, and excess Sema3A disrupts the nephrin/podocin/CD2AP complex (right). b In glomerulonephritis, Sema4D expressed on T-cells interacts with CD72 on dendritic cells to promote T-cell activation, and with CD72 on B-cells to enhance B-cell activation and antibody production (these interactions typically occur in lymphoid organs). An interaction between Sema4D on T-cells and Plexin-B1 on glomerular cells might also be pathophysiologically relevant.](image-url)
podocyte foot processes. It interacts with podocin, a membrane-associated intracellular scaffold, which controls actin dynamics through several proteins, including the multidomain scaffolding protein CD2AP, thereby maintaining podocyte intercellular junctions and podocyte morphology. In mice with podocyte-specific overexpression of Sema3A, or after administration of exogenous Sema3A, nephrin expression is downregulated [6, 10]. Moreover, in immortalized murine podocytes and in isolated glomeruli, Sema3A treatment interferes with the interaction between nephrin and podocin as well as between podocin and CD2AP [5]. Sema3A is known to bind to receptor complexes composed of an A-family plexin and neuropilin-1 [1]. Studies analyzing the expression of these Sema3A receptors have detected Plexin-A1, Plexin-A2 and Plexin-A3 as well as neuropilin-1 in kidney lysates and immortalized cultured podocytes [5, 9] (table 1). Biochemical experiments indicate that Plexin-A1 directly interacts with the cytoplasmic domain of nephrin [6], suggesting that Plexin-A1 could mediate Sema3A effects on the nephrin-podocin-CD2AP complex. In addition, Plexin-A1 interacts with the actin disassembly factor MICAL-1 in immortalized cultured podocytes, and knockdown of MICAL-1 renders podocytes insensitive to a Sema3A-induced contraction [6, 8]. In diabetic mice overexpressing Sema3A in podocytes, podocyte-specific genetic deletion of Plexin-A1 ameliorates diabetic nephropathy, strongly suggesting that Plexin-A1 on podocytes mediates adverse Sema3A-induced effects [8].

Semaphorin 4D in Glomerulonephritis

Glomerulonephritis, an inflammation of the renal corpuscle, is one of the most common causes of kidney disease and often leads to chronic renal failure. Different forms of glomerulonephritis can be classified according to their pathogenesis, their histological features and their clinical course. Sema4D is expressed by T-cells as well as in kidney tubules [12–14] (table 1), and global inactivation of the Sema4D gene has beneficial effects in different mouse models of glomerulonephritis including attenuated glomerular injury and less deposition of immunoglobulins in the glomeruli [12, 15] (fig. 2b). On a systemic level, these mice show reduced formation of antigen-specific antibodies, lower proliferation of antigen-specific lymphocytes and decreased T cell responses [12, 15]. It has been suggested that the adverse effects of leukocyte-derived Sema4D in glomerulonephritis are mediated by its receptor, Plexin-B1, expressed in glomeruli [12]. However, functional evidence for this has not been reported so far. It is likely that the role of Sema4D in glomerulonephritis relies, at least to a significant extent, on its function in the immune system, where it mediates communication between T-cells and dendritic cells as well as between T-cells and B-cells through its alternative receptor CD72 [1, 15] (fig. 2b). A humanized anti-Sema4D monoclonal antibody that interferes with the binding of Sema4D to Plexin-B1 and CD72 is currently in clinical trials for several indications [1] and might have beneficial effects in glomerulonephritis.

Semaphorins and Plexins in Acute Kidney Injury

Acute kidney injury is a very frequent and challenging clinical problem associated with high morbidity and mortality, particularly in intensive care unit patients [16]. As a consequence of injury, tubular epithelial cells (TECs) die from necrosis or apoptosis, which triggers dedifferentiation and proliferation of surviving TECs to eventually restore tubular integrity and function. This repair process is critically influenced by an inflammatory response in the interstitium surrounding the tubules [16]. Several semaphorins have been shown to be crucially involved both in inflammatory processes promoting tubular injury as well as in epithelial repair processes that are required for restoration of kidney function.

Sema3A in Acute Kidney Injury

In normal mouse kidneys, Sema3A is expressed in distal and collecting tubules as well as in podocytes [17, 18] (table 1). Following acute kidney injury induced by transient ischemia or by cisplatin, Sema3A protein expression is upregulated in distal and collecting tubules [17, 18]. Under these pathophysiological conditions, infiltrating immune cells are likely to serve as an additional source of Sema3A in the kidney [18, 19]. The functional role of Sema3A in ischemia/reperfusion injury in vivo has been assessed by both pharmacological as well as genetic approaches employing mice, which carry a loss-of-function mutation in the Sema3A gene. These mice, while having normal tubular morphology and function under physiological conditions, show less tubular injury, decreased apoptosis of TECs and lower serum creatinine levels 1 day after ischemia/reperfusion injury [18] (fig. 3). This is accompanied by a decrease in the renal mRNA expression of pro-inflammatory and pro-apoptotic mediators and less neutrophil infiltration in the kidney [18]. In line with this, prophylactic treatment of mice before ischemia/reperfusion injury with an inhibitor of lipooxygenase or with a recombinant peptide, both of which block Sema3A-in-
duced effects in neurons [11, 20], inhibits apoptosis of TECs and lowers serum creatinine [18]. While the relative significance of the different cell types as sources for Sema3A remains to be dissected, these data establish Sema3A to promote ischemia-induced renal inflammation and tubular injury. The cellular and molecular mechanisms through which Sema3A exerts these effects are not yet entirely clear. Of particular interest is the question on the relevant cell types that are responsive to Sema3A and the identity of the Sema3A receptors expressed by them. Several lines of evidence suggest that dendritic cells and macrophages are a main target of Sema3A in acute kidney injury. Infusion of primary murine dendritic cells, that had been treated with Sema3A for 2 days, prior to ischemia/reperfusion injury increases tubular injury and the mRNA expression of pro-inflammatory mediators in the kidney [18]. Furthermore, Sema3A increases LPS-induced expression of cytokines and chemokines in a macrophage cell line and in primary murine dendritic cells in vitro, suggesting a role of Sema3A in TLR4 signaling in these cells [18]. This would be in accordance with in vivo findings showing that administration of Sema3A aggravates the release of cytokines caused by TLR agonists or bacterial sepsis [19]. In addition to immune cells, Sema3A might directly impact on TECs, as it increases LPS-induced expression of cytokines and chemokines and also aggravates cisplatin-induced apoptosis in these cells in vitro [18]. Which receptors mediate the injury-promoting effects of Sema3A in the kidney is not known to date.

Semaphorin 4B/4D/4G-Plexin-B2 Signaling in Acute Kidney Injury

In addition to class-3-semaphorins, the adult kidney expresses several class-4-semaphorins [13, 14, 21] (table 1). While Sema4A, 4B and 4D are found on renal TECs, Sema4C is expressed in the interstitium, and the localization of Sema4G is not known. One of the receptors of these semaphorins, Plexin-B2, is strongly expressed on renal TECs [13, 21] (table 1). In mice under normal physiological conditions, genetic inactivation of

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**Fig. 3.** Semaphorins and plexins in acute kidney injury. Following acute kidney injury induced by ischemia/reperfusion, some epithelial cells die, and an inflammatory response is induced. Early after injury, Sema3A appears in the urine, where it could serve as a biomarker. Surviving TECs divide in parallel to the epithelial plane to restore epithelial architecture. In the absence of functional Sema3A, tubular injury and apoptosis are decreased, and injury-induced inflammation in the kidney is attenuated. In the absence of Plexin-B2 or its ligands Sema4B, 4D and 4G, surviving epithelial cells fail to align their divisions with the epithelial plane, resulting in tubular obstruction. Box on lower left: an epithelial cell-cell contact is magnified, showing the interaction between Plexin-B2 and Sema4B, 4D and 4G. KO = Knockout.
any of these semaphorins alone, of Sema4B, 4D and 4G together, or of Plexin-B2 specifically in renal TECs, has no impact on kidney morphology and function [13]. After ischemia/reperfusion injury however, Sema4B/4D/4G-triple-deficient and Plexin-B2-deficient mice display striking abnormalities in kidney repair [13]. Following the ischemic insult, surviving TECs of these mice do not show a clear preference for cell divisions parallel to the basement membrane, and cells divide perpendicular instead of parallel to the epithelial plane (fig. 3). This eventually leads to multi-layering of the tubular epithelium and to complete tubular occlusion with loss of renal function [13] (fig. 3). Experiments employing a series of transgenic mice carrying subtle mutations in the Plexin-B2 gene that interfere with particular Plexin-B2-mediated signaling pathways, and of mice with an inducible, TEC-specific inactivation of the small GTPase Cdc42 combined with in vitro experiments indicate that Plexin-B2 controls cell division orientation through its GTPase-activating protein (GAP) domain and regulation of Cdc42 activity [13]. The precise molecular mechanisms that link Sema4B/4D/4G-Plexin-B2 signaling to the spindle orientation machinery to correctly align cell divisions during kidney repair remain to be determined.

**Semaphorins and Plexins as Biomarkers in Kidney Diseases**

For decades, the diagnosis of acute kidney injury has been based on glomerular filtration failure that leads to the rise of the filtration markers creatinine and urea in the blood. However, these markers typically do not accumulate in the blood until hours or even days after injury. This imposes the need for new, sensitive and specific biomarkers that allow for earlier diagnosis and therapeutic intervention. While Sema3A is absent from the urine of normal mice, it becomes detectable after ischemia/reperfusion- or cisplatin-induced injury many hours before creatinine levels elevate in the serum [17]. Studies in humans have shown that these preclinical findings in mice also hold true in pediatric patients undergoing cardiopulmonary bypass surgery, where Sema3A levels in the urine rise as early as 2 h after surgery, while serum creatinine increases significantly only after 48 h [17]. Similar results were obtained in adult patients in various other clinical settings of acute kidney injury [22, 23]. Also in chronic diseases affecting the kidney, urine Sema3A has been shown to serve as an indicator of disease severity. This has been demonstrated for diabetic nephropathy in several drug-induced and genetic mouse models of diabetes and in diabetic patients [9], as well as in hypertensive patients with chronic kidney disease [24]. Of note, in patients suffering from systemic lupus erythematosus, serum Sema3A levels have been reported to inversely correlate with disease activity and the presence of lupus nephritis [25]. The potential use of Sema3A as a biomarker is currently investigated in 2 observational clinical trials: UMIN000013422, enrolling patients with renal diseases, and NCT02406716, enrolling patients with heart failure.

**Concluding Remarks**

Work of the last decade has delineated crucial functions of the semaphorin–plexin system in various mouse models of kidney diseases. It has become clear that semaphorin–plexin signaling plays diverse roles in renal pathophysiology with particular semaphorins and plexins exerting beneficial and others having adverse effects. The data obtained in preclinical mouse models suggest that the semaphorin–plexin system represents a novel and promising pharmacological target in kidney diseases, and future work will have to clarify whether these findings can be translated to humans. Moreover, preclinical and clinical data show that Sema3A has the potential to serve as a biomarker in renal diseases, particularly as an early indicator of acute kidney injury. Given the versatile roles of its numerous members in diverse cell types, additional functions of the semaphorin–plexin system in renal pathophysiology are likely to emerge in the future.

**Disclosure Statement**

T.W. holds a patent on B-type plexin antagonists and uses thereof.

**References**


