

## Review

# Sphingosine 1-Phosphate in Renal Diseases

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## Key Words

Sphingosine 1-phosphate • Kidney disease • Diabetic nephropathy • Glomerulonephritis • Ischemia-reperfusion injury • Wilms tumor

## Abstract

Because of its highly bioactive properties sphingosine 1-phosphate (S1P) is an attractive target for the treatment of several diseases. Since the expression of sphingosine kinases as well as S1P receptors was demonstrated in the kidney, questions about the physiological and pathophysiological functions of S1P in this organ have been raised. In this review, we summarize the current state of knowledge about S1P-mediated functions in the kidney. A special focus is put on S1P modulated signal transduction in renal glomerular and tubular cells and consequences for the development and treatment of several kidney diseases, diabetic nephropathy, glomerulonephritis, ischemia-reperfusion injury, as well as for Wilms tumor progression.

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## Introduction

The sphingolipid molecule sphingosine 1-phosphate (S1P) was first reported as signaling molecule by Ghosh et al. [1] who proposed that sphingosine is intracellularly converted to S1P, which in turn triggers an enhanced calcium mobilization from a calcium pool including an inositol trisphosphate sensitive pool. In parallel, the group of Sarah Spiegel showed that exogenously added sphingosine is able to enhance proliferation of Swiss 3T3 fibroblasts [2] which was later specified to be due to the intracellular conversion of sphingosine to S1P [3]. Indeed, a sphingosine kinase (SK) activity had already been characterized many years

before in blood platelets [4]. To date, two subtypes of SK, SK-1 and SK-2, and several splice variants of each subtype, have been cloned and partially characterized (reviewed in [5]). These subtypes are ubiquitously expressed but show differential biochemical properties and subcellular localizations. Therefore, it is very likely that S1P generated by the two enzymes at different subcellular sites may exert different cellular functions. S1P mainly acts as an extracellular ligand for specific cell surface receptors, the S1P receptors (S1PRs), which belong to the superfamily of G protein-coupled receptors. Five subtypes of these receptors have been identified, denoted S1P<sub>1-5</sub>, which trigger various cell responses depending on the cell type, including proliferation, migration and survival. Moreover, several intracellular targets for S1P were recently identified, indicating novel modes of action for this lipid second messenger [6, 7]. Because of its highly bioactive properties S1P is an attractive target for the treatment of several diseases. Since the expression of SKs as well as S1PRs was demonstrated in kidney lysates as well as in isolated renal cells [8-13], questions about the physiological and pathophysiological functions of S1P in this organ have been raised. Here, we summarize the current state of knowledge about S1P-mediated functions in the kidney. A special focus is put on S1P modulated signal transduction in renal cells and consequences for the development and treatment of several kidney diseases as well as for renal tumor progression.

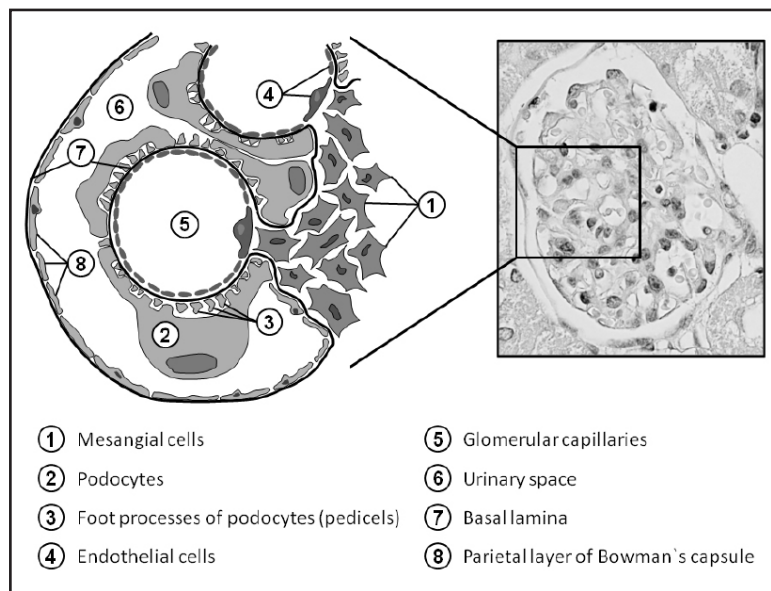
### S1P in renal cells

In the last years, much effort has been undertaken to unravel the cellular functions of S1P in renal cells. Most of these studies have focused on cells isolated from renal glomeruli, including glomerular visceral epithelial cells (podocytes), mesangial cells (MC) and glomerular endothelial cells (Fig. 1), which play important roles in the pathogenesis of several renal diseases including diabetic nephropathy (DN) and various forms of glomerulonephritis (GN). Other studies have focused on the effect of S1P on tubular cells which are thought to be critically involved in the pathogenesis of renal ischemia-reperfusion-induced injury (IRI).

#### *Glomerular cells*

Glomerular MC are smooth muscle-like cells located in the intercapillary space of the glomerulus. Physiologically, they participate in the regulation of the glomerular filtration as well as in the preservation of structural integrity of the glomerulus (reviewed in [14-16]). In addition, in many forms of glomerular diseases MC are activated and show altered migration and proliferation, increased production of extracellular matrix (ECM) components and inflammatory mediators (reviewed in [15-17]). Chen et al. [18] first demonstrated that in cultures of rat MC, S1P as well as sphingosylphosphorylcholine act as exogenous stimuli to trigger increased calcium mobilization and arachidonic acid release through phospholipase C activation. Subsequently, Gennero et al. [11] reported that S1P can either increase proliferation of MC in culture or induce cell death depending on the cell density of the cultures. These authors also characterized for the first time the S1PR expression profile in rat mesangial cells which included the S1P<sub>1-3</sub> and S1P<sub>5</sub> [11]. The proliferative capacity of exogenous S1P on MC was confirmed by Hanafusa et al. [19]. These authors also showed that S1P is neither formed nor released upon cell stimulation with platelet-derived growth factor (PDGF), which is normally secreted from platelets present in damaged or inflamed glomeruli [20]. However, other studies clearly revealed a cross-communication between PDGF and S1P signaling in MC proliferation. Katsuma et al. [10] showed that PDGF up-regulated S1P<sub>2</sub> expression. Furthermore, they suggested the specific involvement of S1P<sub>2</sub> and S1P<sub>3</sub> in MC proliferation. Both receptor subtypes were shown to couple through a pertussis toxin (PTX)-sensitive G protein that resulted in the activation of the classical mitogen activated protein kinase/extracellular signaling regulated kinase (MAPK/ERK) pathway. A connection between PDGF and S1P was also forwarded by Olivera and Spiegel [21] who showed that PDGF can activate SK-1 and induce a nuclear translocation of the enzyme [22]. In this context, S1P was

**Fig. 1.** Cellular and structural components of a renal glomerulus. The immunohistochemical staining (right panel) was generated using a kidney paraffin section stained with periodic acid-Schiff reagent. Counterstaining was performed using Mayer's Hemalaun.



postulated as an intracellular second messenger mediating PDGF-induced proliferation of mouse fibroblasts [21]. Also, Katsuma et al. [10] showed that PDGF induced SK expression and activity as well as intracellular S1P formation in rat MC, leading to the conclusion that SK is at least in part involved in PDGF-induced MC proliferation. Altogether, these studies clearly established a prominent role of the SK/S1P pathway in MC proliferation.

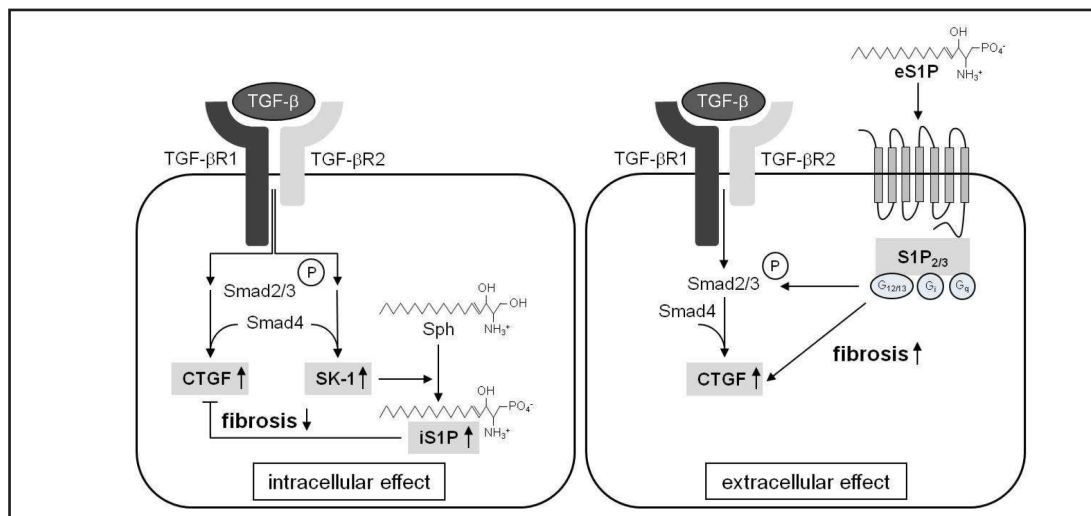
In addition to the proliferative effect, S1P is well known to trigger cell survival. In this context, Hofmann et al. [13] investigated the role of the two SK isoforms, SK-1 and SK-2, in staurosporine induced apoptosis. They showed that renal MCs isolated from SK-2 *knockout* mice were resistant to staurosporine-induced apoptosis whereas the absence of SK-1 led to a more pronounced apoptotic response to staurosporine. These data suggested an opposite function of SK-1 and SK-2 on apoptotic cell responses and were in line with the previous report by Liu et al. [23] who showed that SK-2 is a pro-apoptotic enzyme, which through its BH3 domain binds and traps the anti-apoptotic Bcl-2-family member Bcl-X<sub>L</sub>. Additionally, Igarashi et al. [24] demonstrated that SK-2 is a nuclear protein which inhibits DNA synthesis in various cell lines. In contrast to the poorly understood functions of SK-2 on cell growth and death, the anti-apoptotic function of SK-1 has been more extensively studied and various reports confirmed this hypothesis. In this regards, it was recently shown that certain nephroprotective drugs such as glucocorticoids [25] or PPAR $\gamma$  agonists [26] act at least partially by up-regulating SK-1 expression and activity and consequently increase the cellular S1P level in MC which in turn mediates protection from either stress-induced cell death [25] or fibrotic reactions [26].

An effect of S1P on cell migration of various cell types is also well established. This observation may have impact not only on cancer cell growth, progression and metastasis, but also for renal diseases where migration of MCs is a typical feature like in the early phase of mesangioproliferative glomerulonephritis. Depending on the S1PR subtype expression pattern in different cell types, exogenous S1P can either stimulate (via S1P<sub>1</sub> or S1P<sub>3</sub>) or inhibit (via S1P<sub>2</sub>) migration (reviewed in [27]). In renal MC cultures, it was demonstrated that extracellular S1P, but also the extracellular nucleotides ATP and UTP which act through P2Y purinoceptors, can stimulate MC migration [12]. Down-regulation of SK-1 by siRNA transfection of MC, abrogated the migratory response induced by the nucleotides and remarkably also by S1P [12] suggesting that SK-1 is a key enzyme mediating migration by various extracellular stimuli including S1P itself. This also agrees with the data by Meyer zu Heringdorf et al. [28] who showed that activation of S1PRs enhance SK-1 activity in human embryonic kidney cells (HEK293). These authors speculated that the cells may respond to SK-1 up-regulation/activation by an up- or down-regulation of certain S1PR subtypes.

We recently forwarded the concept that extracellular S1P (eS1P) and intracellular S1P (iS1P) could have opposite effects on certain cell responses such as a fibrotic response. The pro-fibrotic marker connective tissue growth factor (CTGF) is well known to be up-regulated and secreted by transforming growth factor  $\beta$  (TGF- $\beta$ ) *in vitro* and *in vivo* in the course of fibrotic events that are common endpoints of nearly all chronic kidney diseases. The first hint that eS1P affected the pro-fibrotic CTGF came from cDNA microarray analyses of S1P and dihydro-S1P stimulated MC that showed an up-regulation of CTGF mRNA expression by S1P and dihydro-S1P [29]. Later on, Xin et al. [30] demonstrated that also CTGF protein expression and secretion is enhanced by eS1P and forwarded a novel crosstalk activated by S1P and its receptors and the TGF- $\beta$ /Smad signaling pathway. Thus, via one of the S1PRs S1P cross-activated the TGF- $\beta$  receptor complex and thereby acted as a TGF- $\beta$  mimetic in renal MC. A similar mechanism of S1P-triggered Smad activation was later also observed in keratinocytes [31] and in the murine Langerhans cell line XS52 [32]. Furthermore, the mechanism of CTGF up-regulation by S1P seemed not to be restricted to MC, but also occurred in cells of epithelial origin such as in podocytes [33]. In addition, Xin et al. [34] demonstrated that FTY720 and phospho-FTY720 were able to induce CTGF expression in renal MCs via cross-activation of the Smad signaling cascade. Consistent with previously published effects of S1P [30], FTY720 and its derivative phospho-FTY720 induced Smad phosphorylation in a PTX insensitive but suramin dependent manner. Suramin was reported as a selective S1P<sub>3</sub> antagonist when compared to the other S1PR subtypes [35] which made it tempting to suggest that the S1P<sub>3</sub> would be involved in the S1P- and FTY720-triggered Smad cross-activation and subsequent CTGF expression [30, 34]. However, suramin is a rather unspecific drug and many other G protein-coupled receptors were shown to be affected by suramin as well. Therefore, it remains unclear whether S1P<sub>3</sub> is indeed involved in the fibrotic response in renal MC. In this context, Katsuma et al. [36] suggested that S1P<sub>2</sub> and/or S1P<sub>3</sub> are involved in the S1P-induced CTGF expression in renal MCs. More recent data in tumor cells rather suggested the S1P<sub>2</sub> to be involved in the pro-fibrotic effects of eS1P [37]. This conclusion is derived from the finding that phospho-FTY720, which cannot bind to the S1P<sub>2</sub> [38], had no effect on CTGF expression in the Wilms' tumor cell line WiT49 ([37], see below). Furthermore, the S1P<sub>2</sub> antagonist JTE-013 completely blocked the S1P-induced CTGF expression in these cells, whereas the S1P<sub>1</sub> antagonist VPC-44116 did not.

The pro-fibrotic potential of eS1P measured by the up-regulation of CTGF protein expression and secretion was also confirmed for human podocytes [33]. These authors also investigated the role of SK-1 in the expression of CTGF in human podocytes. Interestingly, they could show that the pro-fibrotic cytokine TGF- $\beta$  strongly up-regulated the protein expression and activity of SK-1. Strikingly, this SK-1 activation and generation of iS1P attenuated CTGF expression in podocytes, thus, iS1P rather acted anti-fibrotic. These results were further corroborated by the use of caged S1P which is an inactive S1P derivative that is readily taken up by cells and upon photolysis is cleaved into mature S1P [39]. Caged S1P, when applied to cells and photolysed to generate iS1P, also attenuated CTGF expression [33]. In addition, SK-1 overexpression expected to generate high amounts of iS1P also reduced CTGF expression in the supernatant, whereas a pharmacological inhibitor of SK-1, SKI II (2-(p-hydroxyanilino)-4-(p-chlorophenyl) thiazole), led to a more pronounced CTGF expression upon TGF- $\beta$  treatment. These findings in podocytes were recently also confirmed for MC [26]. These authors demonstrated that thiazolidinediones (TZDs) which are agonists of the peroxisome proliferator-activated receptor gamma, also induce SK-1 expression and activity and the generation of iS1P in renal MC, which was functionally coupled to lower amounts of CTGF. Pharmacological and genetic approaches to inhibit SK-1 abolished this effect *in vivo* and *in vitro*, suggesting that the known anti-fibrotic potential of TZDs is at least partially due to iS1P generation [26]. All these data strongly suggest that targeting of eS1P is a valid approach to prevent fibrotic processes. Alternatively, one may stimulate intracellular SK-1 activity and increase the levels of iS1P. However, this is a double edged sword since iS1P could as well be secreted by still unclear mechanisms and once in the extracellular space it may turn into a pro-fibrotic signal (Fig. 2).





**Fig. 2.** Schematic overview on the opposite effects of intracellular (left panel) and extracellular (right panel) S1P for the expression of pro-fibrotic CTGF in renal glomerular cells. Abbreviations used: CTGF, connective tissue growth factor; S1P, sphingosine-1-phosphate; S1PR, S1P receptor; SK-1, sphingosine kinase 1; Sph, sphingosine; TGF-β, transforming growth factor β; TGF-βR, TGF-β receptor.

Lan et al. [40] reported the up-regulation of SK-1 under high glucose conditions which was paralleled by increased iS1P levels and fibronectin expression in MCs. Surprisingly, SK-1 siRNA and the SK inhibitor *N,N*-dimethylsphingosine (DMS) abolished the high glucose-induced fibronectin expression, proposing a rather pro-fibrotic role of SK-1. By the use of the S1P<sub>2</sub> antagonist JTE-013 the authors further suggested that S1P<sub>2</sub> is involved in this pro-fibrotic response [40], in line with various previous studies highlighting the pro-fibrotic contribution of eS1P and S1P<sub>2</sub>.

#### Tubular cells

Among a wide range of tubular cell lines available, the human HK-2 cell line and the mouse TKPTS proximal tubular cell line are the most widely used cell culture models for current studies to unravel the regulatory function of S1P in IRI based molecular events. The proximal tubule is highly oxygenated under healthy conditions and is associated with a high metabolic rate [41, 42]. Proximal tubular cells represent the primary site of damage upon ischemia/reperfusion and therefore, these cells are considered a suitable model system to study *in vitro* the molecular details of IRI. Immortalized HK-2 cells were derived from normal adult human kidney proximal tubular epithelium by transduction with human papilloma virus 16 E6/E7 [43]. Using this HK-2 cell line, Kim et al. [44] demonstrated that the anesthetic drug isoflurane induced SK-1 mRNA expression as well as SK-1 activity which resulted in increased S1P generation. They also found that *in vivo* in a murine model of IRI, isoflurane protected from renal failure and necrosis and that the protection was lost by SK inhibitor treatment and by a S1P<sub>1/3</sub> antagonist ([44], see below). The protective and anti-necrotic mechanism in HK-2 cells was suggested to include S1P<sub>1</sub>-mediated ERK and protein kinase B/Akt activation and heat shock protein 70 induction [45] as well as release of TGF-β1 [46]. They put forward the hypothesis that an interaction between isoflurane and plasma membranes and particularly caveolar microdomains would lead to TGF-β1 release, which in turn stimulates SK-1 and S1P formation with a subsequent S1P<sub>1</sub> activation to ERK stimulation [47]. A novel therapeutic application for inhalational anesthetics was thus suggested by the authors. A similar protective effect of S1P<sub>1</sub> activation was also reported for the mouse TKPTS cell line [48]. Additionally, overexpression of SK-1 in HK-2 cells also protected against peroxide-induced necrosis and led to enhanced heat shock protein 27 expression that was reversed by S1P<sub>1</sub> antagonism [49]. Finally, Park et al. [50] showed

that the selective S1P<sub>2</sub> antagonist JTE-013 induced SK-1 expression and further attenuated necrosis and apoptosis in HK-2 cells, whereas a S1P<sub>2</sub> agonist had the opposite effect. Indeed, the finding that a receptor antagonist in the absence of the receptor ligand has gene inducing capability is surprising at a first glance and it can presently not be excluded that JTE-013 may have additional cellular effects, e.g. acting as a partial agonist or even unspecifically.

Altogether, these *in vitro* data further established the protective function of S1P in an increasing number of cellular systems.

### S1P in renal disease models

First evidence for the importance of S1P in renal physiology was given by Bischoff et al. [51-53] who showed that intravenous and intrarenal infusions of S1P led to renal vasoconstriction and caused natriuresis in spite of a reduction of renal blood flow ([51-53], reviewed in [54]). The effects were blocked by PTX indicating the involvement of at least one G<sub>i</sub>-coupled S1PR [52]. More than one decade later, Zhu et al. [55] readdressed the involvement of S1PR subtypes in renal function. They demonstrated that intramedullary administration of FTY720 produced an increase in sodium excretion, an effect which was blocked with the S1P<sub>1</sub> antagonist W146. Consequently, the authors suggested that S1P might produce natriuretic effects via activation of S1P<sub>1</sub>, which in turn affects transport mechanisms in the renal medulla [55]. The understanding of S1P signaling in many cellular systems promotes the idea to use this knowledge to treat diverse diseases. To date, various strategies are discussed to either specifically activate or inhibit S1PRs or to deplete S1P from the serum/plasma and thereby to diminish its pathophysiological actions. The usefulness of eliminating plasma S1P levels in the treatment of angiogenic diseases was recently proven by using a neutralizing anti-S1P antibody (sonopizumab®). Humanized S1P antibodies were effective in blocking S1P-mediated release of pro-angiogenic and prometastatic cytokines in cancer models [56, 57] and also reduced injury in a laser-induced retinopathy model [58]. A different approach was recently forwarded by Huwiler et al. [59] who used recombinant S1P lyase from a thermostable bacterium (*Symbiobacterium thermophilum*). This prokaryotic enzyme, in contrast to the eukaryotic enzyme, lacks a transmembrane sequence at its N-terminus and therefore lacks membrane association. Intravenous application of this S1P lyase rapidly degraded plasma S1P levels but the effect was only transient and after 6h, plasma S1P had recovered to normal levels again [59]. When used in a neovascularization model of the developing chicken embryo, the chorioallantoic membrane (CAM) model, recombinant *Symbiobacterium thermophilum* S1P lyase significantly reduced vessel formation triggered by injected tumor cells [59].

Numerous studies not only addressed the function of S1P in renal cells but also in diverse *in vivo* diseases models. In the following part, we will recapitulate the present data generated in situations of DN, GN and IRI.

#### Diabetic nephropathy

Nearly 30% of all diabetic patients develop DN, the major cause of end-stage renal failure, which is characterized by clinical symptoms like albuminuria and declined glomerular filtration rate. Pathological hallmarks of DN are enhanced secretion and deposition of ECM proteins, the expansion of the mesangium and the thickening of the glomerular basement membrane (reviewed in [60]). The enhanced secretion of cytokines and growth factors (e.g. TGF-β, CTGF) and subsequent MC proliferation and ECM accumulation lead to substantial fibrotic changes in the kidney and to the initiation of glomerulosclerosis.

Based on the *in vitro* findings described above, several groups investigated how sphingolipid metabolism is altered under diabetic conditions *in vivo*. A variety of diabetic mouse models are available which however show substantial differences in the renal consequences of diabetes (reviewed in [61]). The application of a single high dose of streptozotocin (STZ) is a widely used and well-established murine model for DN. Its diabetes

inducing effect is based on the toxicity of STZ on pancreatic  $\beta$ -cells and it thereby mimics specifically type 1 diabetes. Using this STZ model in rats, Geoffroy et al. [62] showed that neutral ceramidase and SK activities were significantly up-regulated in isolated glomeruli from 4 days STZ treated rats. They also determined increased S1P levels in the glomeruli although the sphingosine levels were not altered. Derived from these observations, they suggested a possible involvement of sphingolipid metabolites in the glomerular proliferative response in the early stage of DN [62]. In line with this notion, Lan et al. [63] showed that substances that ameliorated alloxan-induced DN in mice such as berberine, also down-regulated SK-1 mRNA and protein expression and S1P levels *in vivo*. Although they did not show a causal link between the SK-1 down-regulation and disease amelioration, they proposed that inhibition of SK1/S1P may be a new therapeutic option for renoprotection in diabetes. The same group recently published that the up-regulation of SK-1 in STZ induced DN in mice was paralleled by enhanced levels of the matrix constituent fibronectin [40]. Further mechanistic studies however were only performed in MC cultures where the authors showed that SK-1 down-regulation or inhibition, or alternative stimulation with exogenous S1P, resulted in reduced fibronectin production. In another study, Ren et al. [33] also demonstrated the up-regulation of SK-1 in STZ-induced DN, but these authors by contrast suggested the up-regulation of SK-1 to be a protective mechanism in the fibrotic process. When using SK-1 knockout mice, they found a more severe STZ-induced disease as measured by higher albuminuria and enhanced CTGF expression in kidney sections when compared to wild type C57BL/6 mice [33]. The mechanism was further investigated in cultures of human podocytes where they could show that TGF- $\beta$  is not only a strong inducer of fibrogenic CTGF, but remarkably also of SK-1. Inhibiting SK-1 by an inhibitor, SKI II, or down-regulating SK-1 by siRNA, led to an amplified CTGF expression, whereas overexpression of SK-1 or using caged-S1P to increase iS1P resulted in reduced CTGF expression. Collectively, these data strongly suggested that the TGF- $\beta$  dependent induction of SK-1 acts like a break in the fibrotic process by impeding CTGF expression [33]. Most critical seemed to be the site of action of S1P, either intracellularly to promote an anti-fibrotic effect, or extracellularly to trigger a pro-fibrotic response [30, 33] (Fig. 2).

However, the S1PR subtype involved in this pro-fibrotic eS1P action is still unclear. By using receptor antagonists both, the S1P<sub>2</sub> and S1P<sub>3</sub> have been forwarded as candidates ([30, 40], see above). In a recent study, Imasawa et al. [64] reported an unbalanced mRNA expression profile of S1P<sub>2</sub> versus S1P<sub>1</sub> in diabetic glomeruli in that the ratio of S1P<sub>2</sub>/S1P<sub>1</sub> was increased in renal glomeruli of rats one year after STZ injection. Considering the *in vitro* findings of Lan et al. [40], i.e. that S1P-stimulated fibronectin expression involves S1P<sub>2</sub> in MC cultures, the up-regulated mRNA expression of S1P<sub>2</sub> in diabetic glomeruli may well point to a contribution of this receptor subtype in the pathogenesis of DN. Beside this possible disease-promoting effect of S1P<sub>2</sub>, a protective effect of chronic S1P<sub>1</sub> activation has been suggested. Thus, Awad et al. [65] showed that STZ-induced DN in rats was reduced by either the unselective S1PR agonist FTY720 or the selective S1P<sub>1</sub> agonist SEW2871. Since SEW2871 in contrast to FTY720, did not reduce circulating lymphocytes, they suggested that the renoprotective effect occurred independent of lymphocytes. Also, the protective effect of FTY720 was still observed in S1P<sub>3</sub> knockout mice excluding this receptor subtype in the renoprotective mechanism [65]. Overall, these studies indicated that at least the so far discussed receptors, S1P<sub>1</sub> and S1P<sub>2</sub>, might be attractive targets to be further considered in the treatment of DN.

### Glomerulonephritis

GN is a huge group of diverse inflammatory kidney disease that irrespective of their initial triggering cause may end in end-stage renal failure. GN is divided into several subclasses depending on the clinical disease context, the progression, acute or chronic, and the histological and immunological changes involved (reviewed in [66]). A hallmark of many forms of GN is the activation of MC leading to increased MC proliferation, inflammatory mediator synthesis and ECM production. Because of their prominent role

in the pathogenesis of GN, cultured MCs have become an attractive model system to study the molecular mechanisms in GN. The impact of S1P on these events *in vitro* has been discussed above. However, to date no study addressed the role of S1P in the pathogenesis of GN *in vivo*. A few studies investigated the effect of the immune modulator FTY720 on the progression of experimental anti-Thy1.1-induced GN. In both acute and chronic anti-Thy1.1-induced GN in rats, FTY720 was shown to have a beneficial effect [67, 68]. It not only reduced lymphocyte infiltration but also reduced proteinuria, tubulointerstitial matrix expansion, TGF- $\beta$ 1 and fibronectin expression. These authors suggested that the elimination of lymphocytes from the circulation and consequently also from the kidney, is the critical protective event triggered by FTY720. However, to date, it is controversially discussed how important lymphocytes are in the pathogenesis of the different forms of GN. Along this line, Lebleu et al. [69] showed evidence that lymphocytes are dispensable for GN but required for interstitial fibrosis. Moreover, Sui et al. [70] recently published that FTY720 acts protective in the model of anti-glomerular basement membrane-induced GN in mice, which could be mediated by FTY720-dependent down-regulation of S1P<sub>1</sub>, S1P<sub>2</sub> and S1P<sub>5</sub> in the spleen although the exact mechanisms remained unclear. Taken together, these studies indicate that FTY720 might be an attractive approach for the treatment of both acute and chronic GN. Moreover, Wenderfer et al. [71] showed that KRP-203, a specific agonist for S1P<sub>1</sub> and S1P<sub>4</sub> [72], decreased kidney injury and improved survival in a model of lupus nephritis (MRL/lpr mice). Those mice spontaneously develop a systemic autoimmune disease, characterized by lymphocyte accumulation in the kidney, which in turn leads to a mesangioproliferative GN and glomerulosclerosis (reviewed in [73]). Administration of KRP-203 reduced the number of infiltrating T-cells and circulating lymphocytes and therefore attenuates renal injury [71]. Again, this study stresses the idea that activation of S1P<sub>1</sub> has beneficial effects in lupus nephritis and maybe also other chronic cell-mediated autoimmune diseases.

#### *Ischemia-reperfusion injury*

RI is an inescapable event in kidney transplantation often leading to acute kidney injury (AKI), which is directly linked to prolonged hospitalization, and increased morbidity and mortality (reviewed in [74]). Overall, AKI is associated with a wide range of changes in mechanisms such as intense immune responses, coagulation, generation of oxidative stress and hypoxia, and apoptosis. Since many cell culture studies have suggested the involvement of sphingolipids in these cell responses, a contribution of these lipids also in the pathogenesis of IRI seems obvious. The catabolic and metabolic routes of these lipids as well as their signaling pathways are considered attractive targets to interfere with IRI and progression to AKI. The first evidence that sphingolipids play a role in post-ischemic acute renal injury was presented by Zager et al. [75] who showed that in mice subjected to renal ischemia, a drop in cortical sphingosine and ceramide levels occurred which either normalized again after reperfusion as seen for sphingosine or even rose to supranormal levels as detected for ceramide [76, 77]. Paradoxically, acid and neutral sphingomyelinase activities both decreased in an unremitting fashion during the ischemia/24 h-reperfusion period [77] stressing the hypothesis that ceramide not only derives from sphingomyelin but also from the de-novo synthesis pathway. Recently, Jo et al. [78] used SK-1 and SK-2 deficient mice to investigate whether the conversion of ceramide/sphingosine to S1P has an effect on renal ischemia/reperfusion injury. Theoretically, depletion of SK-1 or SK-2 should lead to increased sphingosine and eventually also ceramide levels. They found no effect of SK-1 depletion on the extent of injury, but they observed more severe injury in SK-2 deficient mice [78] leading the authors to conclude that SK-2 is a protective enzyme. Opposite to this, Park et al. [49] found enhanced IRI in SK-1 deficient mice, but reduced IRI in SK-2 deficient mice. Moreover, Kim et al. [44] used the volatile anesthetic isoflurane to activate SK-1 and generate S1P that, in turn, via activation of S1P<sub>1</sub> protected mice from renal failure in a murine model of IRI. In SK-1 deficient mice, no protection by isoflurane occurred any more [44]. Also, the two SK-1 inhibitors DMS and SKI II abrogated the nephroprotective effect of isoflurane [44, 49]. The reason for the discrepancy between the results of Jo et al. [78] and Park et al.



[49] remained unclear but could be due to differences in the experimental setup, e.g. both groups used a different model of AKI as well as mice from different sources. However, SK deficient mice were also used in a myocardial ischemia/reperfusion injury model. In this model, it was shown that both SK-1 and SK-2 deficient mice exerted a more severe myocardial injury upon ischemia/reperfusion treatment when compared to wild-type mice [79, 80]. Altogether, these studies cannot give a conclusive answer about the functions of SK-1 and SK-2 in ischemia/reperfusion injuries, and clearly more studies are needed to clarify the contribution of these enzymes in ischemic injury models. More consistent are data about an IRI protective function of the product S1P. This hypothesis was further strengthened by various studies using the immunomodulatory agent FTY720. Thus, Troncoso et al. [81] demonstrated that FTY720 prevented ischemic reperfusion damage in a rat kidney model. Since FTY720 in its active phosphorylated form is an unselective S1PR agonist, acting on S1P<sub>1</sub> and S1P<sub>3-5</sub>, the question came up which receptor subtype would mediate this protective effect of FTY720. Awad et al. [82] showed that the protective effect of FTY720 is due to a selective S1P<sub>1</sub> activation since the S1P<sub>1</sub> antagonist VPC-44116 reversed the protective effect of FTY720 on mouse IRI. In addition, the S1P<sub>1</sub> selective agonist SEW2871 exerted a similar protective effect as FTY720 on IRI [82, 83]. Whether the nephroprotective effect of FTY720 is only due to the depletion of peripheral lymphocytes or due to a direct effect on kidney cells is still a matter of debate. On the one side, Awad et al. [82] suggested that FTY720 acted via lymphocyte depletion. Moreover, Lai et al. [84] reported that SEW2871 prevented T cell infiltration, which also has been implicated in the pathogenesis of IRI [85] by inhibiting T cell egress from lymphoid tissue in an early stage. In contrast to these studies, Bajwa et al. [48] showed a lymphocyte independent nephroprotective effect of FTY720. Treatment with either FTY720 or SEW2871 reduced injury in both Rag-1 knockout mice, deficient for T and B lymphocytes, as well as in wild-type mice [48]. These results fit very well with their previous observation that the S1P<sub>1</sub> antagonist VPC-44116 reversed FTY720-mediated nephroprotection [82]. As described above, this lymphocyte-independent protective effect was supported by *in vitro* findings that S1P<sub>1</sub> agonists reduce cell death of mouse kidney proximal tubule epithelial cells induced by LPS- or H/R through the involvement of the ERK and Akt pathways ([48], see above). In addition, Lee et al. [86] also showed a protective effect of the S1P and S1P<sub>1</sub> pathway in hepatic IRI-induced AKI by preventing endothelial cell injury in mice, which was proposed to occur independent of lymphocyte homing [86]. Opposite to these studies that support the involvement of S1P<sub>1</sub>, Jo et al. [78] showed that both kidney function and the reduced vascular permeability in IRI were preserved in S1P<sub>3</sub><sup>-/-</sup> mice suggesting the involvement of this receptor subtype in IRI and consequently a protective effect of S1P<sub>3</sub> antagonism. Regarding the source of S1P that can contribute to the protective effect in IRI, Sola et al. [87] proposed that especially macrophages, that undergo apoptosis due to IRI, release S1P which, in turn, acts in an autocrine manner on macrophage S1P<sub>3</sub> to release neutrophil gelatinase-associated lipocalin (NGAL/Lcn-2) and triggers increased proliferation of tubular epithelial cells and thereby prevents from IRI-induced cell death [87]. Besides this protective effect of macrophage S1P<sub>3</sub>, the authors also showed that the S1P<sub>1</sub> mediates a renal protection against IRI that is independent of macrophages which is in line with several previous studies [48, 82]. Furthermore, it was shown that the S1P<sub>2</sub> agonist SID46371153 exacerbated renal IRI, whereas the S1P<sub>2</sub> antagonist JTE-013 protected against renal IRI [50]. In terms of mechanism, these authors showed that inhibition of S1P<sub>2</sub> led to Rho kinase and HIF-1 $\alpha$  activation, SK-1 up-regulation, S1P generation and activation of S1P<sub>1</sub> that consequently triggered renoprotection. However, caution is required when making conclusions solely based on pharmacological inhibitors such as W146 and JTE-013 since the selectivity pattern of these compounds is limited and unspecific effects cannot be excluded.

#### *S1P and Wilms tumor progression*

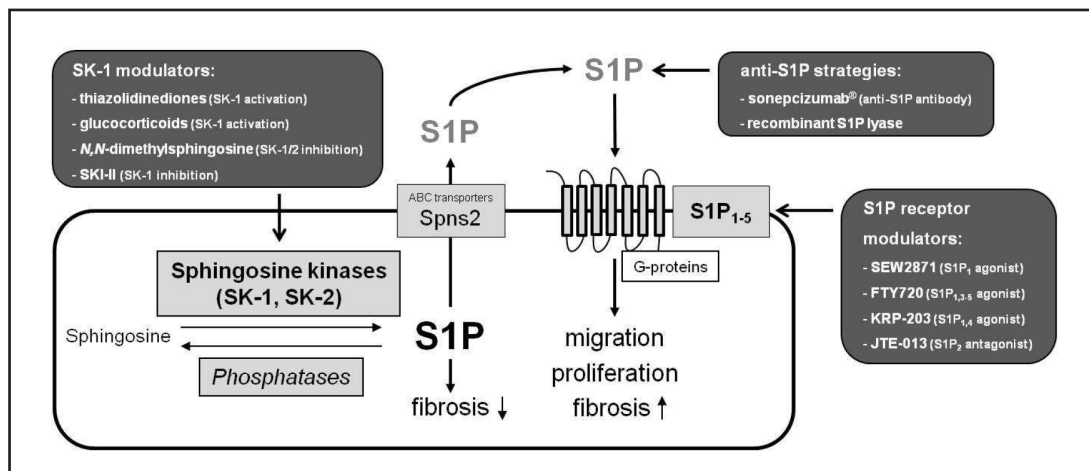
Wilms tumor or nephroblastoma is an embryonic cancer of the developing kidney that is considered to derive from renal stem or progenitor cells that show a failure in

differentiation. Wilms tumor is the most prevalent renal malignant tumor in children. But fortunately, it is also one of the most treatment-responsive tumors (reviewed in [88]). Thus, several therapeutic options are available to treat Wilms tumor successfully. Since plenty of evidence exists that supports a key role for S1P in tumor growth and progression both *in vitro* and *in vivo* (reviewed in [89]), it is tempting to speculate that S1P may also play a crucial role in Wilms tumor pathogenesis. By microarray analysis of advanced Wilms tumor tissue, Zirn et al. [90] identified a series of differentially expressed genes that could play a role in disease progression. Among these genes, CTGF was found to be down-regulated in advanced Wilms tumors. Furthermore, the treatment of Wilms tumor cell lines with all-trans retinoic acid, a potential therapeutic that inhibits cell proliferation and induces cell differentiation [91], normalized CTGF expression again [92]. These data led the authors to suggest a negative regulatory role for CTGF in Wilms tumor progression. However, the generality of CTGF being a negative regulator of tumor development is not given. In this view, CTGF has also been described as tumor promoter in various cancers including acute lymphoblastic leukemia, breast, cervical, hepatocellular and pancreas cancers, but as tumor suppressor in chondrosarcoma, lung and colorectal cancers (reviewed in [93]).

Li et al. [94] showed that in the Wilms tumor cell line WiT49, S1P up-regulated CTGF expression and subsequently inhibited cell proliferation which further confirmed the negative regulatory role of CTGF on Wilms tumor growth. The S1P-triggered effect on CTGF was mediated by the S1P<sub>2</sub> [94]. In addition, the overexpression of S1P<sub>2</sub> reduced the S1P-stimulated migration of Wilms tumor cells [95] consistent with other studies on various other cancer cell lines that also showed a negative role of S1P<sub>2</sub> in cell migration (reviewed in [27]). However, it should be kept in mind that S1P<sub>2</sub> may also signal to other genes. In this context, Li et al. [96] demonstrated that in WiT49 cells, the S1P/S1P<sub>2</sub> axis can also induce cyclooxygenase-2 (COX-2) expression and leads to enhanced prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) formation. However, PGE<sub>2</sub> is known to mediate growth and progression of various tumor types by promoting angiogenesis and inhibiting apoptosis (reviewed in [97]) and it was shown that COX-2 inhibition significantly suppressed tumor growth even *in vivo* in an orthotopic xenograft model of human Wilms tumors in mice [98]. Thus, whether S1P<sub>2</sub> activation or inhibition is more favorable in treating Wilms tumors remains uncertain.

### Conclusions and future perspectives

The discovery that S1P can act in a dual way either from the extracellular space as ligand to cell surface S1PRs, or as a second messenger in the intracellular compartments to regulate a multitude of cellular processes including cell proliferation, survival, migration and modulation of gene transcription sometimes in an opposing manner, has triggered a huge research effort to understand the molecular mechanisms of S1P action and to develop strategies to regulate intracellular and extracellular S1P levels. The availability of suitable tools like genetically modified mice, low molecular mass inhibitors and highly potent receptor agonists and antagonists with improved properties will be helpful in clarifying these processes. In the past, the development of selective S1PR subtype agonists and antagonists seemed to be most attractive. Indeed, first data display a renoprotective effect of S1PR agonists, e.g. SEW2871 (S1P<sub>1</sub>) and KRP-203 (S1P<sub>1,4</sub>), and antagonists, JTE-013 (S1P<sub>2</sub>), in various animal models of renal diseases such as diabetic nephropathy and glomerulonephritis (Fig. 3). In this context, especially the development and market introduction of FTY720 (fingolimod, Gilenya®) for the treatment of multiple sclerosis has undoubtedly proven the therapeutic relevance of targeting S1PRs and has strongly boosted research on sphingolipids in various other inflammatory and proliferative diseases including kidney diseases. Beside S1PR-specific strategies, the effectiveness of S1P trapping in serum/plasma by a neutralizing anti-S1P antibody (e.g. sonpicipumab®) or S1P degradation by the use of recombinant S1P lyase to diminish its pathophysiological actions needs to be evaluated. In addition, several



**Fig. 3.** Possible pharmacological interventions into sphingolipid metabolism for the treatment of renal diseases. Abbreviations used: ABC transporters; adenosine tri-phosphate binding cassette transporters; S1P, sphingosine 1-phosphate; Spns2, spinster homolog 2.

studies reveal that targeting SK-1 seems to be a worthwhile strategy to treat renal diseases (Fig. 3). However, some controversial data about the beneficial effect of either activation of SK-1 and subsequent formation of iS1P or its inhibition by SKI-II or DMS make clear that, regarding kidney diseases, the picture of S1P and its involvement in the pathogenesis of such diseases is still controversial. Therefore, the usefulness of targeting the SK/S1P/S1PR signaling device in kidney diseases awaits further clarification in future studies.

### Conflict of Interest

The authors declare no conflict of interest in writing this article.

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