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COMMENTARY

Renal mesangial cells: moving on sphingosine kinase-1

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Sphingosine kinase-1 (SphK1) catalyses the phosphorylation of sphingosine to sphingosine-1-phosphate (S1P), which acts on at least five specific G-protein-coupled receptors and also intracellularly. SphK1 has been implicated in cell proliferation, cancer growth, chemoresistance, immune cell functions and cell migration. In this issue of the *British Journal of Pharmacology, Klawitter et al.* demonstrate that extracellular nucleotides stimulate the migration of renal mesangial cells. The nucleotides furthermore upregulated SphK1 expression and activity, and this enzyme was required for nucleotide-induced migration. Together with previous findings, these data raise exciting questions: by which mechanism does SphK1 regulate migration in mesangial cells, how is the interplay of purinoceptors and S1P receptors organized in these cells, and how would SphK1-deficient mice respond to kidney damage?
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Abbreviations: MAPK, mitogen-activated protein kinase; S1P, sphingosine-1-phosphate; SphK, sphingosine kinase

In renal mesangial cells, extracellular nucleotides acting on purine P2Y receptors stimulate several signalling pathways, thereby promoting proliferation (via p42/44 mitogen-activated protein kinase (MAPK)), stimulating prostaglandin release (via phospholipase A_2) and protecting from apoptosis (via Akt) (see Huwiler *et al*., 2002; Klawitter *et al*., 2007 and citations therein). In this issue of the *British Journal of Pharmacology,* Huwiler's group (Klawitter *et al*., 2007) demonstrates that extracellular adenosine triphosphate and uridine triphosphate stimulate the migration of renal mesangial cells, and they suggest that this might be an important event in the repair mechanism during inflammatory kidney diseases. Furthermore, the nucleotides upregulated expression and activity of sphingosine kinase-1 (SphK1), and this enzyme was essential for nucleotide-induced mesangial cell migration (Klawitter *et al*., 2007).

Sphingosine kinases catalyse the phosphorylation of sphingosine to sphingosine-1-phosphate (S1P), which is an ubiquitous lipid mediator, regulating cell growth and survival, migration and adhesion (for reviews, see Spiegel and Milstien, 2003; Sanchez and Hla, 2004; Chalfant and Spiegel, 2005). S1P acts on at least five specific G-protein-coupled receptors ($S1P_{1–5}$) and also intracellularly. Recently, the role of S1P in lymphocyte trafficking, angiogenesis and vascular permeability has gained particular attention as the novel immunosuppressant, FTY720, modulates these conditions by interfering with S1P receptors (Brinkmann *et al*., 2004; LaMontagne *et al*., 2006). Two sphingosine kinases, SphK1 and SphK2, and transcriptional variants thereof, have been identified in mammals so far. SphK1 and SphK2 are differentially expressed and appear to have opposing cellular functions, with SphK2 acting preferentially as a pro-apoptotic agent, whereas SphK1 has been implicated in cell proliferation, cancer growth, chemoresistance, immune cell functions and cell migration (for reviews, see Spiegel and Milstien, 2003; Taha *et al*., 2006).

The formation of S1P by sphingosine kinases is a highly regulated process. Both transcriptional and post-transcriptional regulation of SphK1 activity and/or protein have been reported in several cellular systems (for reviews, see Spiegel and Milstien, 2003; Taha *et al*., 2006). Acute stimulation of sphingosine kinase activity by $P2Y_2$ receptors, leading to S1P formation within a timescale of minutes, has been shown before (Alemany *et al*., 2000). Klawitter *et al*. now demonstrate a specific activation of SphK1, but not SphK2, by purine receptors in mesangial cells. Activation of the enzyme was biphasic, with a rapid phase, most likely during to post-transcriptional activation, and a delayed phase of transcriptional upregulation of SphK1 within hours, which was mediated by protein kinase C and MAPK. This was similar, for example, to epidermal growth factor-stimulated SphK1 expression in human breast cancer cells (Döll *et al*., 2005) and histamine- and hepatocyte growth factor-stimulated SphK1 expression in endothelial cells (Duan *et al*., 2004;

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Huwiler *et al*., 2006). SphK1 was essential for nucleotide-stimulated migration. This was demonstrated using (1) short interfering RNA (siRNA) to SphK1 and (2) mesangial cells from SphK1-deficient mice. Interestingly, mice deficient in SphK1 were viable and fertile with no obvious dysfunctions (Allende *et al*., 2004). Therefore, considering the present data, it would be important to study the response to renal damage in these mice, for example by streptozotocin treatment, which in a recent study was found to cause upregulation of sphingosine kinase activity and increased S1P levels within renal glomeruli (Geoffroy *et al*., 2005).

Another interesting observation was that deletion of SphK1 did not affect nucleotide-induced proliferation of mesangial cells. As discussed by the authors, mesangial cells differ in this regard from many other cell types in which SphK1 is essential for cell growth. SphK1 has furthermore been implicated in induction of cyclooxygenase-2 (reviewed in Taha *et al*., 2006); therefore, it would be interesting to determine whether purinoceptor-stimulated prostaglandin production is affected by SphK1 deletion.

The differential cellular response to SphK1 deletion raises the intriguing question, how does SphK1 mediate migration of mesangial cells in response to extracellular nucleotides? In several cellular systems, activation of SphK1 leads to autocrine S1P release and activation of G-protein-coupled S1P receptors. This was shown, for example, in mast cells, in which crosslinking of Fcε receptor-I stimulated SphK1 activity, S1P secretion and internalization of $S1P_1$ and $S1P_2$ receptors, with $S1P_1$ mediating migration and $S1P_2$ degranulation of the cells in response to antigen (Jolly *et al*., 2004). SphK1-siRNA had no effect on S1P-induced chemotaxis in this study. In PC12 cells and dorsal root ganglion cells, SphK1 was required for nerve growth factor-induced neurite extension, and SphK1 plasma membrane translocation and $S1P_1$ cross-activation were involved in this process (Toman *et al*., 2004). Remarkably, Klawitter *et al*. also showed that deletion of SphK1 abrogated cell migration induced by extracellular S1P. This highly interesting observation suggests that either the SphK1 protein or its catalytic product, S1P or a shift in the ceramide/sphingosine/S1P-balance acted intracellularly for signalling migration. In this scenario, extracellular S1P would then activate and induce SphK1. Indeed, G-protein-coupled S1P receptors can stimulate sphingosine kinase activity (Meyer zu Heringdorf *et al*., 2001). However, intracellular targets of S1P remain to be identified.

It has been found that G-protein-coupled S1P receptors differentially regulate migration, with $S1P_1$ and $S1P_3$ stimulating and $S1P_2$ inhibiting this response (Sanchez and Hla, 2004). Therefore, assuming that S1P inside-out signalling is involved in SphK1-dependent migration of mesangial cells, it is possible for the cells to modulate their response to SphK1 upregulation in a specific manner by up- and downregulating S1P receptors. Mesangial cells express the S1P receptors, $S1P_1$, $S1P_2$ and $S1P_3$ (Katsuma *et al*., 2002), which mediate increases in intracellular Ca^{2+} concentrations, activation of MAPK, p38, JNK and Akt kinases, cross-activation of transforming growth factor-β receptor and activation of the Smad signalling cascade (see Xin *et al*. (2004a) and citations therein). Furthermore, S1P stimulated proliferation (via MAPK activation), inhibited apoptosis (Katsuma *et al*., 2002) and induced migration (Klawitter *et al*., 2007) in these cells. Interestingly, purinoceptors are known to interfere with S1P receptor signalling in mesangial cells, causing a heterologous desensitization of S1P-stimulated MAPK activation (Xin *et al*., 2004b). As SphK1 deletion had no effect on purinoceptor-stimulated proliferation, it might be speculated that the heterologous desensitization serves to shut off certain S1P-receptor signalling pathways that otherwise would be activated by inside-out signalling, including MAPK activation and eventually inhibition of migration by $S1P_2$, thereby facilitating other pathways such as pro-migratory signalling by $S1P_1$ and/or $S1P_3$. Taken together, the data presented by Klawitter *et al*. demonstrate differential, cell type-specific signalling by SphK1. Together with previous findings, these data raise challenging questions regarding the mechanism of SphK1-mediated migration and the interplay of purine and G-protein-coupled S1P receptors in mesangial cells.

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