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Minireview

Sphingosine-1-phosphate: dual messenger functions

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Abstract The sphingolipid metabolite sphingosine-1-phosphate (S1P) is a serum-borne lipid that regulates many vital cellular processes. S1P is the ligand of a family of five specific G protein-coupled receptors that are differentially expressed in different tissues and regulate diverse cellular actions. Much less is known of the intracellular actions of S1P. It has been suggested that S1P may also function as an intracellular second messenger to regulate calcium mobilization, cell growth and suppression of apoptosis in response to a variety of extracellular stimuli. Dissecting the dual actions and identification of intracellular targets of S1P has been challenging, but there is ample evidence to suggest that the balance between S1P and ceramide and/or sphingosine levels in cells is an important determinant of cell fate.

Key words: Sphingosine-1-phosphate; EDG-1; Sphingosine kinase; Cell growth; Apoptosis

1. Introduction

Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid metabolite formed by the phosphorylation of sphingosine by sphingosine kinase. Like many other sphingolipids, S1P was long thought to be mainly a degradative metabolite of sphingolipids formed during turnover of eukaryotic cell membranes. However, since our discovery over 10 years ago that S1P plays an important role in cell growth regulation [1], it has been implicated in many and diverse biological processes, such as cell growth, differentiation, cell survival, angiogenesis, cell migration (reviewed in [2–5]), and more recently, in the regulation of immune function by influencing mast and T cell functions and lymphocyte trafficking [6–8]. With the discovery that S1P can bind to specific cell surface G protein-coupled receptors, the concept emerged that S1P can function as an extracellular first messenger and perhaps as an intracellular second messenger, and these dual functions as well as five specific receptors may explain the diverse biological processes reported to be regulated by this lipid mediator. This review is focused on the inside–outside functions of S1P.

2. Extracellular actions of S1P: first messenger functions

In the last few years, interest in the role of S1P has accelerated with the finding that S1P is the natural ligand of the orphan G protein-coupled receptor EDG-1 (now called $S1P_1$) [9,10], and with the subsequent identification of four other family members with high specificity for S1P and dihydro-S1P: $S1P_2$, $S1P_3$, $S1P_4$ and $S1P_5$ (reviewed in [3]). These G protein-coupled receptors are differentially expressed in different tissues, and couple to a variety of G proteins that regulate various signal transduction pathways. As a result, S1P can potentially stimulate diverse signal transduction pathways in different cell types as well as within the same cell, resulting in the possibility of diverse biological outcomes, depending on the cell type, G proteins that are present, and the pattern of S1P receptor (S1PR) expression (Fig. 1).

One of the more widely studied functions of extracellular S1P is the regulation of cell migration and its role in angiogenesis. S1P stimulates directed migration of endothelial cells [11] and vascular smooth muscle cells [12,13], critical events in the formation and extension of new blood vessels, as well as promoting capillary-like tube formation by bovine aortic endothelial cells [11]. These events appear to be mediated primarily by the binding of S1P to $S1P_1$ and the subsequent activation of a pertussis toxin-sensitive G_i protein. The role of S1P and $S1P_1$ in angiogenesis has been further substantiated by disruption of the *s1p₁* gene in mice. These mice die at embryonic day 13.5 from hemorrhage due to incomplete maturation of the vascular system [14]. Moreover, fibroblasts isolated from these embryos fail to migrate in response to platelet-derived growth factor (PDGF) or S1P and fail to activate the small GTPase Rac, known to be involved in cell migration. Collectively, these data reveal an important role for extracellular S1P and $S1P_1$ in vascular maturation. In addition to endothelial and smooth muscle cells, S1P also acts as a chemoattractant for hematopoietic precursor cells [15] and immature dendritic cells [16], raising the possibility that S1P may control the recruitment of inflammatory cells to sites of inflammation and help modulate the immune response. Indeed, recent studies have shown that lymphocyte trafficking is altered by S1P and by a sphingosine kinase-produced phosphorylated metabolite of the immunosuppressive agent FTY720 [7,8]. Phosphorylated FTY720 was a high affinity agonist of at least four of the five S1P receptors and induced emptying of lymphoid sinuses and inhibition of egress into lymph. These observations have important clinical implications for sphingolipids in immunosuppression [7,8].

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Like $S1P_1$, $S1P_3$ appears to be involved in stimulation of cell migration mediated by S1P and regulation of cytoskeletal rearrangements and membrane ruffling associated with cell motility [17–19]. In addition to its intracellular anti-apoptotic role, binding of S1P to $S1P_3$ has also been shown to enhance survival by suppression of Bax expression and activation of endothelial nitric oxide synthase, phosphatidylinositol 3-kinase and Akt [19–21]. Contrary to the stimulatory effects of $S1P_1$ and $S1P_3$, activation of $S1P_2$ inhibits cell migration [17]. That S1P can both stimulate and inhibit cell migration at first appears contradictory, but the net effect of S1P on cell migration may depend on the relative levels of receptor expression, receptor turnover, and S1P concentrations. Indeed, at low concentrations, S1P induced smooth muscle cell migration but at higher concentrations it was inhibitory [12]. Another effect of S1P is the induction of neurite retraction and neuronal cell rounding, mediated through $S1P_3$ [22]. Much less is known about the function of $S1P_5$. It is, however, highly expressed in oligodendrocytes and astrocytes [23] and S1P inhibits extracellular signal-regulated kinase (ERK) activation and proliferation in cells overexpressing $S1P_5$ [24]. It is therefore possible that S1P/$S1P_5$ signaling may play an important role in nervous system development.

Activation of a number of signaling pathways attributed to extracellular S1P may account for some of the observed biological effects. The activation of the small GTPases Rac and Rho [17,25] has been linked to cytoskeletal rearrangements and motility. Other relevant signaling pathways include activation of the ERK and p38 mitogen-activated protein kinases [26], intracellular calcium mobilization, and activation of phospholipase D and Akt.

The existence of multiple signaling pathways induced by S1P and the expression of multiple S1PRs might suggest some redundancy in S1P functions. The lethality of $S1P_1$-null mice indicates that $S1P_1$ has unique functions not shared by other S1PRs. Moreover, it was recently shown by the use of single and double $S1P_2$/$S1P_3$-null mice that $S1P_2$ and $S1P_3$ share some functions, such as activation of Rho, but that $S1P_3$ alone may play a major role in calcium mobilization [27]. Interestingly, although surviving $S1P_2$/$S1P_3$-null mice appeared to develop normally, litter sizes were greatly reduced and there was increased perinatal death, suggesting redundancies in $S1P_2$ and $S1P_3$ functions vis-à-vis perinatal development.

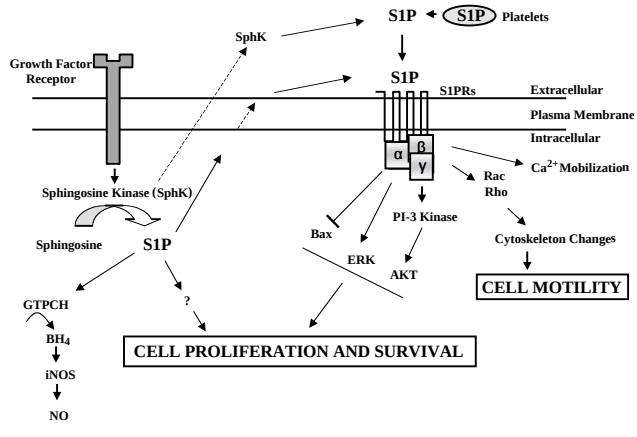

Fig. 1. Major signaling pathways and functions of intracellularly generated and extracellular S1P. GTPCH, GTP cyclohydrolase; BH_4, tetrahydrobiopterin; iNOS, inducible NO synthase; NO, nitric oxide.

Recently, we showed that in C6 astroglioma cells, intracellular S1P plays a role in the regulation of tumor necrosis factor-α (TNF-α)-induced activation of GTP cyclohydrolase expression and synthesis of tetrahydrobiopterin, a co-factor required for nitric oxide synthase activity [28]. Surprisingly, we found that, although C6 cells can secrete S1P, which is enhanced by TNF-α, treatment of C6 cells with exogenous S1P or dihydro-S1P had no effect on tetrahydrobiopterin biosynthesis. However, both S1P and dihydro-S1P markedly stimulated ERK1/2 in C6 cells, which express cell surface S1P receptors. Interestingly, although this ERK activation was blocked by PD98059, which also reduced cellular proliferation induced by enforced expression of sphingosine kinase, PD98059 had no effect on GTP cyclohydrolase activity [28]. Collectively, these results suggest that only intracellularly generated S1P plays a role in regulation of GTP cyclohydrolase activity and tetrahydrobiopterin levels. This is one of the few studies that clearly show a distinct difference between intracellular and extracellular actions of S1P.

3. S1P is a second messenger

Studies from our lab and many others have implicated S1P as a second messenger in cellular proliferation, cell survival and suppression of apoptosis (Fig. 1, reviewed in [29]). Furthermore, a variety of growth factors and cytokines, including PDGF, epidermal growth factor, TNF-α, and nerve growth factor, which are well known inducers of cellular proliferation and/or differentiation, also activate sphingosine kinase, the enzyme that forms S1P from sphingosine, and thereby increasing cellular S1P levels [30–32]. An early clue that S1P may play a role as a second messenger mobilizing calcium from internal sources independently of inositol trisphosphate arose with the finding that sphingosine derivatives generated inside cells stimulated the release of calcium [33].

In many of the initial studies, S1P was added exogenously to elevate cellular levels, and although S1P can be rapidly taken up by cells, this approach led to some confusion because it was difficult to determine the site of action of S1P. With the cloning of sphingosine kinase and the development of specific molecular tools to increase intracellular levels of S1P, some evidence has surfaced to suggest that intracellular S1P plays a key role in cell growth and survival [34]. Moreover, specific inhibitors of sphingosine kinase also inhibit cell proliferation and survival induced by various stimuli as well as sphingosine kinase overexpression (reviewed in [35]).

The intracellular targets of S1P remain much more elusive. Although no direct targets have yet been conclusively identified, there are some provocative clues for future searches. Microinjection of S1P into fibroblasts increases DNA synthesis [9] and calcium mobilization from internal stores [36]. PDGF induces translocation of sphingosine kinase to the nuclear envelope with a concomitant increase in nucleus-associated sphingosine kinase activity [37]. This implies that S1P may have a role in the nucleus, and it was suggested that it may be involved in cell cycle progression, although no direct evidence for this has yet appeared.

S1P has also been shown to activate ERK [38] and inhibit c-Jun N-terminal kinase (JNK) activation [39], which is significant since the balance of ERK and JNK activation has

been implicated in the control of apoptosis [40]. Moreover, ceramide, another sphingolipid metabolite that induces apoptosis in many cell types (reviewed in [41]), opposes the effects of S1P on these pathways [39]. This, as well as the opposing effects of S1P and ceramide on the induction of apoptosis, has led to the model in which the dynamic balance of S1P and ceramide determines the fate of the cell. More recently, elevated sphingosine levels in mast cells have been shown to inhibit allergic activation and production of leukotrienes, whereas elevated S1P levels resulted in activation of mast cells and increased leukotriene production [42], supporting the notion that the balance of intracellular sphingolipid metabolites controls many biological responses. As would be expected for an intracellular signaling molecule, the levels of S1P in cells are low and tightly regulated by the balance between sphingosine kinase-dependent synthesis and degradation by an endoplasmic pyridoxal phosphate requiring lyase and by phosphohydrolases. Recently, a specific S1P phosphohydrolase was cloned and characterized [43]. Overexpression of this S1P-degrading enzyme decreased cellular S1P levels, increased sphingosine and ceramide, and promoted apoptosis, in further support of the model in which the dynamic balance of S1P and ceramide determines the fate of the cell [43].

4. Does S1P signal inside-to-outside, outside-to-inside, or both?

As discussed above, it is now well accepted that extracellular S1P is an important mediator of many physiological processes. The early studies demonstrating that PDGF activated sphingosine kinase, thereby increasing intracellular S1P levels, implicated a role for intracellular S1P in cell survival, proliferation and the inhibition of apoptosis [30,39]. However, the findings that extracellular S1P can inhibit apoptosis [20,21], stimulate proliferation of mesangial cells [44], and inhibit proliferation of hepatic myofibroblasts [45] have raised the possibility that S1P may have simultaneous dual functions in many of these biological processes (Fig. 1). Our recent discovery that PDGF-directed cell motility requires cross-talk from the PDGF receptor to $S1P_1$ via activation of sphingosine kinase and formation of S1P led us to put forward the idea that intracellular S1P can either be secreted or diffuse across the plasma membrane and activate cell surface S1PRs in an autocrine or paracrine manner [13]. PDGF also stimulates and induces translocation of sphingosine kinase to the leading edge of migrating cells [46], resulting in the formation of a steep, extracellular S1P gradient at the leading edge of a migrating cell, thereby directing cell movement. Together these data suggest an inside-to-outside signaling paradigm whereby an agonist induces intracellular production of S1P, which then stimulates its receptor present on the same cell. This mode of action makes the elucidation of intracellular vs. extracellular S1P signaling pathways even more challenging. Some other complicating factors are that sphingosine kinase can also be secreted by endothelial cells [47], suggesting that S1P generated directly in the extracellular space may contribute to cell migration and angiogenesis, and extracellular S1P may even stimulate its own intracellular production [48].

Transport of S1P in and out, as well as within cells has received little attention. The finding that the cystic fibrosis transmembrane regulator (CFTR), a member of the ABC family of transmembrane transporters, is also a S1P transporter that can regulate the uptake of S1P may provide a mechanism by which S1P can cross the plasma membrane in a regulated manner [49]. Although it is not known whether CFTR can also mediate secretion of S1P, this transporter could provide a mechanism by which cells modulate extracellular S1P signaling through its internalization.

In conclusion, a better understanding of S1P signaling pathways, whether intra- or extracellular, may prove to be useful in identifying targets for the development of therapeutics for a number of disease states. There is much interest in the development of S1P antagonists or sphingosine kinase inhibitors for the treatment of cancer since S1P plays such an important role regulating cell proliferation, survival, migration and vascularization, all critical processes in cancer cell biology. Of note, S1P is a lipid component of both high density and low density lipoproteins and may play a role in atherogenesis and heart disease, although it is as yet unclear whether S1P is a pro- or anti-atherogenic mediator (reviewed in [50]). Modulation of S1P signaling may also prove to be useful in therapies for asthma and allergy. A challenging task still at hand is the conclusive demonstration of intracellular actions independent of known S1PRs and identification of intracellular targets of S1P.

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