Sphingosine-1-Phosphate and the Intestine
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S1P Biosynthesis and Signaling

Sphingosine-1-phosphate (S1P) is a ubiquitous sphingolipid metabolite that is constitutively present in a wide array of human tissues and body fluid. Historically thought to be merely a structural component of the plasma membrane, S1P has gained considerable attention over the past two decades as a pleiotropic bioactive signaling molecule [1,2]. Since the earliest discoveries that S1P regulates cell growth [3,4] and apoptosis [5], a plethora of research has now established the pivotal role S1P plays in regulation of a multitude of critical cellular processes. S1P has been implicated in cell proliferation and survival, cell migration and invasion, cytoskeletal rearrangement, regulation of calcium homeostasis, immune cell trafficking, angiogenesis and vascular maturation, as well as endothelial and epithelial barrier function. The diverse biological functions of S1P are accredited to its ability to function both intracellularly and extracellularly as a second messenger ligand through global S1P receptors.

Cellular S1P levels are tightly regulated by the dynamic activity of its synthetic and degradative enzymes. S1P is produced by ATP-dependent phosphorylation of sphingosine by the enzymatic activity of sphingosine kinases, of which there are two known isoforms, sphingosine kinase 1 (Sphk1) and sphingosine kinase 2 (Sphk2). S1P can be subsequently dephosphorylated back to sphingosine by two specific S1P phosphatases (SPP1 and SPP2) or be irreversibly degraded by S1P lyase (SPL) [6,7]. Both Sphk1 and SPL have garnered recent attention as attractive therapeutic targets in regulation of S1P levels and signaling and will be discussed in further detail later in this review. Activation of Sphk1 by a variety of stimuli, including vascular endothelial growth factor (VEGF) [8], platelet derived growth factor (PDGF) [9], insulin-like growth factor (IGF)[10], TNF-α[11], and IL-1β[12], elicits translocation of the enzyme to the plasma membrane, where the enzymatic activity of Sphk1 produces S1P that then acts either intracellularly or is exported out of the cell via ABC transporters[1].

S1P exerts its actions in a complex signaling pathway commonly termed “inside-out” signaling (Figure 1). S1P is known to operate through both intracellular and extracellular signaling. Intracellular S1P acts as a 2nd messenger and has been shown to lead to calcium release and proliferation[4,13]. Alternatively, many of the well described actions of S1P actions are attributed to receptor-mediated signaling through one of five high affinity G protein-coupled S1P receptors (S1PR1-5). The S1P can then exert its effects extracellularly in a paracrine manner via binding to one of these receptors, thus eliciting a G protein-dependent signaling cascade[14].

S1P, its receptors, and associated enzymes are ubiquitous, but differentially expressed across multiple tissues and cell lines[15]. Interestingly, S1P tissue levels are relatively low compared to plasma levels, and a significant concentration gradient exists that has been purported to regulate lymphocyte trafficking [16]. The concentration of plasma S1P varies from 0.2 to 0.9 µM as compared to markedly lower S1P tissue levels ranging from 0.5 to 75 pmol/mg [17]. The source of plasma S1P has historically been attributed to platelets due to their high Sphk activity and lack of S1P lyase [18]. However, recent studies have challenged that school of thought, instead implicating both red blood cells and endothelial cells as the major cellular sources of plasma S1P [19]. Increases in S1P production have been linked to an array of pathologic conditions, such as carcinogenesis and metastasis, atherogenesis and diabetes [2,20]. A wealth of research has also demonstrated the protective effects of S1P in a variety of pathophysiologic processes including myocardial infarction, stroke, graft versus host disease, and acute lung injury/ARDS [21-24]. In this review, we summarize the current role of S1P and its regulatory enzymes in intestinal function and pathophysiology, related specifically to colon carcinogenesis, inflammatory bowel disease, and intestinal barrier function.

S1P and Colon Carcinogenesis

The concept of the sphingolipid rheostat, which postulates that cell fate is determined by the dynamic balance between S1P and its precursor ceramide, has gained considerable attention within the cancer biology world. Whereas ceramide mediates induction of cell apoptosis and senescence [25,26], S1P has been shown to regulate oncogenic processes such as proliferation, migration and angiogenesis [27]. Thus, the elegant balance of sphingolipids in regulation of both cell survival and programmed cell death has garnered a multitude...
of further research into their role in carcinogenesis and possible chemotherapeutic targets. Furthermore, S1P has been implicated in the initiation and progression of a wide array of human cancers including breast cancer, melanoma, prostate cancer, hepatocellular carcinoma, lung cancer, pancreatic cancer as well as head and neck cancers [28]. In this review, we will focus on the role S1P, its precursors, and regulatory enzymes play in colon carcinogenesis.

The earliest study linking sphingolipids and colon cancer was published in 1986 in which Dudeja et al. [29] demonstrated that treatment with a chemical colonic carcinogen, 1,2-dimethylhydrazine (DMH), induced increased expression of sphingomyelin in rat colonic mucosa. Subsequently, a wealth of studies followed that further demonstrated that administration of dietary sphingomyelin and other sphingolipid derivatives proved to be protective in colon carcinogenesis and tumorigenesis [30-32]. Greenspon et al. [33] recently reported that intestinal epithelial cells exposed to S1P are protected against apoptotic stimuli. Similarly, a recent study demonstrated that dietary sphingomyelin resulted in reduced colonic tumor formation and progression in DMH-treated mice both when fed before or following tumor initiation [34]. Thus, dietary sphingomyelin proved to play a novel and encouraging role in both the treatment and prevention of colon cancer in a small animal model of DMH-induced colon cancer. Despite these interesting findings, much of the research linking colon carcinogenesis to the S1P axis has focused upon the roles of the regulatory enzymes, Sphk and SPL, in colon carcinogenesis.

Sphingosine kinase (Sphk) was first identified as a potential oncogene when overexpression of human Sphk1 in fibroblast cells resulted in malignant transformation and tumor formation [35]. Since this early study, others have explored the relationship of Sphk and colon cancer by utilizing different small animal models of colon carcinogenesis. Firstly, the Min mouse model of multiple intestinal neoplasia (Apc<sup>min/-</sup> mice) relies upon genetic mutation of the tumor suppressor gene APC, and in these mice it was seen that deletion of the Sphk1 gene in Apc<sup>min/-</sup> mice resulted in a significant decrease in adenoma size, however no difference in adenoma incidence as compared to Apc<sup>min+/-</sup> Sphk1<sup>-/-</sup> mice [36]. Subsequently, multiple studies followed demonstrating the role of Sphk as an oncogene in colon carcinogenesis. Recent literature established that Sphk1 is significantly up-regulated in both human colorectal cancer tissue specimens as well as in the colon carcinogenic, azoxymethane (AOM)-induced aberrant crypt foci (ACF) and tumors in the murine colon [36,37].

Although these results indicated that Sphk1 likely played a critical role in tumor progression, it did not indicate whether it participated in the initiation of intestinal tumors [36]. A follow-up study expanded on these results by demonstrating that Sphk1 expression in human metastatic primary colon cancers was significantly higher than in non-metastatic primaries, thus further delineating a role for Sphk1 in both tumor progression and metastasis [20]. Interestingly, in an AOM-induced colon cancer model, Sphk1 Knockout mice, but not Sphk2 knockout mice, demonstrated a significant decrease in the incidence and multiplicity of ACF, the pre-malignant colon cancer lesions [20]. Thus, these results support the hypothesis that Sphk1 plays a more pivotal role than Sphk2 in colon carcinogenesis. To challenge this theory, the effect of a selective Sphk2 inhibitor, ABC294640, was evaluated in a colitis-driven model of colon cancer. Interestingly, oral administration of ABC294640 to mice exposed to dextran sulfate sodium (DSS) to induce colitis and AOM resulted in a significant attenuation in both the incidence and multiplicity of macroscopic tumors and microscopic dysplastic lesions [38]. The authors conclude that an interplay exists between the Sphkisoenzymes, contributing to the regulation of colon carcinogenesis.

S1P lyase (SPL), another S1P regulatory enzyme, has recently received attention in colon carcinogenesis research. As previously mentioned, SPL is responsible for the irreversible degradation of S1P. Previous research has demonstrated that SPL is highly expressed in normal intestinal and colonic epithelium, where it serves to prevent S1P accumulation following digestion of dietary sphingolipids [39]. However, a recent study has shown that S1P expression is significantly down-regulated in human colon cancer tissue samples as compared to normal adjacent tissue [40], thereby allowing S1P accumulation and potentiating cell proliferation, tumorigenesis and angiogenesis. Additionally, the Min murine model study also demonstrated a significant reduction in S1P expression in adenomatous polyps as compared to a greater than 2-fold higher expression in surrounding normal tissue [40]. Thus, although in its infancy, research examining the role of the Sphk/SPL/S1P pathway in colon carcinogenesis has promising potential to produce novel chemotherapeutic targets in both the prevention and treatment of colon cancer in the near future.

**S1P and Inflammatory Bowel Disease**

Inflammatory bowel disease (IBD), a disease entity consisting of Ulcerative Colitis (UC), Crohn’s disease, and indeterminate colitis, carries a high disease burden in Western countries where the incidence ranges from eight to fifteen people per 100,000 [41]. Although the exact etiology of IBD is unknown, it is postulated to involve a complex interaction between dysregulated inflammatory responses, mucosal barrier disruption, exposure to infectious agents, and genetic disposition. Histologically, IBD is typified by mucosal inflammation and ulcers, as well as elevated colonic levels of inflammatory cytokines, most prominently a dramatic increase in TNF-α levels [42,43]. Accordingly, the role of S1P in the regulation of immune cell trafficking and TNF-α signaling has been well-established [6]. Recent studies with Sphk-knockout mice and experiments with S1P-mimetics in animal models of colitis delineate a role for S1P in IBD.

Recent literature demonstrated an increase in expression of Sphk1 in the colonic epithelial cells in patients with active UC, thereby indicating that the Sphk1/S1P pathway is aberrantly activated in human IBD [44]. Sphk1<sup>-/-</sup> mice were partially protected against dextran sulfate sodium (DSS)-induced colitis as evidenced by attenuation in colonic shortening, mucosal disruption, and weight loss [44]. Furthermore, an oral non-selective Sphk inhibitor demonstrated encouraging results in treatment of both acute and chronic DSS-induced colitis in mice with a significant attenuation in both colonic shortening and influx of inflammatory mediators [45].

Further research defining the role of S1P in IBD has examined the treatment of murine colitis with two distinct S1P receptor modulators, fingolimod (FTY720) and KRP-203. The immunosuppressive drug FTY720 is a non-selective S1PR agonist that induces downregulation and degradation of S1PR on lymphocytes, thus blocking lymphocyte trafficking to the site of inflammation [46]. Recent literature has demonstrated that FTY720 was effective in treatment of colitis in both DSS-induced colitis and the CD4<sup>+</sup>CD62L<sup>-</sup> T cell transfer model of colitis [47]. Additionally, interleukin-10 gene-deficient (IL-10<sup>-/-</sup>) mice, who develop a Crohn’s-like colitis by three months of age, had a significant reduction in their severity of colitis with FTY720 treatment [48]. FTY720 was demonstrated to ameliorate the effects of induced colitis through reduction of both circulating peripheral blood lymphocytes and migration of lymphocytes to the site of inflammation in the colonic lamina propria. A similar improvement of colitis in IL10<sup>-/-</sup> mice was demonstrated following treatment with selective S1P<sub>1</sub> agonist.
KRP-203, by decreasing lymphocytic infiltration in the colonic lamina propria [49]. These studies and others underscore the role of the Sphk/SIP pathway in the pathogenesis of IBD and as potential targets for therapeutic intervention. However, to date, no clinical trials with S1P modulators for the treatment of IBD have been undertaken.

S1P and Intestinal Barrier Function

In contrast to the aberrant activation of the SIP pathway in colon carcinogenesis and IBD, SIP has been demonstrated to exhibit protective effects in a growing number of tissues including the heart, liver, brain, kidney, and most notably the lung [21,50-52]. Current literature on endothelial barrier function demonstrates that SIP enhances endothelial barrier function and attenuates acute lung injury in animal models through cytoskeletal strengthening and enhancement of adherens junction proteins [53]. Upregulated SIP is protective in the lung and heart in ischemia-reperfusion and improves survival after lipopolysaccharide exposure [22,53,54].

Despite the abundance of knowledge about SIP’s enhancement of endothelial barrier integrity, only recently has the role of SIP in intestinal epithelial barrier integrity been investigated. Greenspoon et al. [55] recently demonstrated that treatment with SIP augments intestinal barrier function by decreasing paracellular permeability in differentiated rat intestinal epithelial cells. Treatment with SIP resulted in a dose-dependent increase in expression of adherens junction protein, E-cadherin, as well as enhanced cortical redistribution of the junctional protein. Additionally, intestinal epithelial cells with upregulated expression of SpHK1 show increased levels of several barrier proteins such as claudin-1 and occludin. However, further research to define the in vivo role of SIP in intestinal epithelial barrier integrity is ongoing.

Conclusions

SIP has shown great promise in the last decade as a modulator of apoptosis, lymphocyte trafficking, cell growth, barrier enhancement, and survival upon exposure to ischemia-reperfusion or septic conditions. More specifically, SIP and its regulatory enzymes have been shown to be upregulated in the lung and heart in ischemia-reperfusion and improve survival upon exposure to ischemia-reperfusion or septic conditions. Although much of the research is ongoing, SIP’s regulatory enzymes have been shown to be upregulated in the lung and heart in ischemia-reperfusion and improve survival upon exposure to ischemia-reperfusion or septic conditions.

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References


