Of Spiders and Crabs: The Emergence of Lysophospholipids and Their Metabolic Pathways as Targets for Therapy in Cancer

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Abstract

Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P), two small lysophospholipids, are potent inducers of many of the hallmarks of cancer including cell proliferation, survival, migration, invasion, and neovascularization in vitro and in vivo tumor models. Furthermore, the enzymes metabolizing LPA and S1P and their receptors are aberrant in multiple cancer lineages and exhibit transforming activity altering patterns and targets for metastasis. Several recent studies show the remarkable activity of new chemical genomics and/or potential novel drugs in preclinical models. Combined with the physiologic and pathophysiologic activities of LPA and S1P, these studies suggest the implementation of preclinical and clinical evaluation of LPA and S1P as therapeutic targets.

Background

Lysophosphatidic acid. Lysophosphatidic acid (LPA) is a small bioactive phospholipid produced normally by activated platelets, fibroblasts, mesothelial cells, and adipocytes and abnormally by some cancer cells, creating an autonomous signaling environment (1, 2). LPA is linked to a variety of physiologic and pathophysiologic activities including wound healing (3), production of angiogenic factors (4), chemotaxis (5, 6), neo-intima formation (7), tumor-cell invasion (8), metastasis (9–11), and cell cycle progression (12). Although alterations in blood levels remain controversial, LPA is highly elevated in the peritoneal ascitic fluid of patients with ovarian cancer (13), being produced by tumor cells as well as by mesothelial cells in the peritoneal cavity, suggesting an active role in the progression of ovarian cancer. In cancer, LPA seems to function mainly as a survival factor and less as a growth and proliferative agent, with much of its apparent growth factor activity relating to increased cell survival. LPA has the ability to induce substantial migration and invasion as well as the production of other lipids (12), to alter membrane fluidity and possibly as a ligand for PPARγ, a nuclear transcription factor receptor involved in metabolic function (29, 30), and potentially as a ligand for PPARγ, a nuclear transcription factor receptor involved in metabolic function (31).

Sphingosine 1-phosphate. Activated platelets produce another species of homologous lysosphospholipid, sphingosine 1-phosphate (S1P). This molecule has diverse signaling functions including being required for angiogenesis (16), cardiac development (17), immune responsiveness (18), and, like LPA, cell proliferation (19), wound healing (3), and the suppression of apoptosis (20). Immunoneutralization of S1P produces dramatic inhibition of the ability of tumors to establish a network of blood vessels and provide the tumor with a constant supply of resources (21). Whether S1P is a penultimate mediator of the effects of multiple proangiogenic factors or whether it is permissive for angiogenic responses remains to be determined (22).

LPA receptors. The biological functions of LPA are mediated by several G protein–coupled receptors, LPA1, LPA2, and LPA3 (23–26), numbered to sequentially represent the order of their discovery. PPARγ, GPR23/LPA4 (27), and GPR92/LPA5, a newly identified receptor expressed primarily in the heart, lymphocytes, and small intestine (28), are under investigation to determine whether they are bona fide and functionally relevant LPA receptors. Intracellular LPA functions as a precursor for the production of other lipids (12), to alter membrane fluidity (29, 30), and potentially as a ligand for PPARγ, a nuclear transcription factor receptor involved in metabolic function (31). Intracellular PPARγ seems to mediate some of the effects of LPA on vascular function, potentially contributing to the development of vascular plaques (32). The G protein–coupled receptor LPA4 was de-orphaned after it was reported to bind LPA, but it is not widely expressed in human tissues (27), unlike the endogenously expressed LPA1-LPA3 receptors which appear in different combinations in virtually every tissue in the body. Further studies are necessary to establish the GPR92/LPA5 receptor’s role in LPA-mediated signaling, but it is intriguing that this receptor is also highly expressed in embryonic stem cells, implying a role in embryonic development (33).

S1P receptors. Unlike the tissue-specific expression of LPA receptors, the G protein–coupled receptors that bind S1P are ubiquitously expressed in the human body (34). S1P has five transmembrane receptors, S1P1 to S1P5, which with the LPA receptors, constitute the Edg family of receptors. These cell surface receptors mediate the extracellular actions of S1P, although S1P has additional receptor-independent intracellular functions.
**LPA metabolism.** The phosphodiesterase autotaxin (ATX), formerly known as lysophospholipase D (35–37), which exacerbates tumorigenesis and tumor aggressiveness, plays a central role in lysophospholipid metabolism contributing to the production of LPA, and to a lesser degree, to S1P production (38). As ATX knockout mice have profound vascular defects and LPA levels are decreased in heterozygous mice, ATX plays a critical role in neovascularization and is a major mediator of LPA production (39). First discovered as a secreted motility inducing protein in the medium of melanoma cells (40), ATX hydrolyzes the choline head group from lysophosphatidylcholine (LPC) or sphingosylphosphorylcholine to yield LPA and S1P, respectively (35, 41). These lysophospholipids can then bind extracellularly to ATX and inhibit its activity. Thus, LPA are S1P are able to regulate their own biosynthesis by preventing the hydrolysis of their precursor lipids (42). This observation was critical to the identification of useful ATX inhibitors (43).

**SIP Metabolism.** S1P production occurs primarily through the action of sphingosine kinase 1 (Sphk1) and sphingosine kinase 2 (Sphk2), isoforms that phosphorylate the precursor molecule sphingosine, converting it to S1P. Sphk regulates multiple functional outcomes including intracellular calcium release, angiogenesis, antiapoptosis, chemotaxis, neutrophil priming, activation of immune effector cells and inflammation enhancement. (44) Analogues for sphingosine such as N,N-dimethylsphingosine, inhibit Sphk (45), providing strong chemical genomics and potential lead compounds for drug development.

**Clinical and Translational Advances**

**Crabs.** The inherent signaling properties of LPA and S1P suggest that both could contribute to the etiology of cancer by enhancing tumor growth, survival, vessel formation, and metastatic potential; however, despite strong circumstantial evidence, direct proof of a role in tumor development and as a therapeutic target had been lacking. In spite of this, a number of recent reports using chemical genomics, which may eventually show usefulness as therapeutic drugs, targeting the production and action of LPA and S1P, have shown a direct relevance to both cancer pathobiology and therapeutic targets.

LPA became associated with ovarian cancer after the discovery of its elevation in ascitic fluid and malignant effusions of women with ovarian cancer (46, 47). It may be involved in other cancers because of its synthesis by ATX, which is up-regulated in multiple tumor lineages and because of its degradation by Lipid phosphate phosphatases, which are down-regulated in many cancers (48). Indeed, the manipulation of either ATX or Lipid phosphate phosphatases markedly alters tumor growth, survival, and motility in vitro and in vivo (49). Furthermore, deregulated expression of LPA2 or LPA3 receptors appears as malignant transformation transpires in cancers of the ovary (50), colon (51, 52), breast (53), intestine (54), and glioma (55). Strikingly, overexpression of the LPA receptors or ATX is transforming, resulting in the increased frequency of tumorigenesis and, in particular, in altered metastatic capabilities and targets.

In a similar manner, many studies have suggested that Sphk1 plays a role in tumorigenesis by increasing the concentration of S1P, affecting the dynamically balanced sphingolipid rheostat (56), and thereby increasing the survival of cancer cells (57). Sphk1 is associated with cancers in the prostate (58, 59), colon (60), and potentially, breast (61, 62). In addition, the expression of Sphk1 also correlates with poor survival in some brain cancers (63). Finally, the role of Sphk2 is less clear, yet Sphk1 functions as an oncogene being sufficient to transform cells (64).

**Spiders.** In addition to cancer, LPA and S1P are central to the pathobiology of a wide variety of disease processes ranging from spider bites (65, 66), diarrheal toxins (67), vascular plaque (32), transplantation rejection (18), and immune diathesis. The most intriguing of these is the recent demonstration that the production and action of LPA is required for the toxic effects of envenomation by the Loxosceles reclusa or brown recluse spider (Fig. 1). Remarkably LPA receptors are required for cells to be susceptible to the pathogenic effects of sphingomyelinase D, the enzyme responsible for the devastating symptoms (42). It was named sphingomyelinase D to reflect its ability to cleave sphingomyelin into choline and ceramide 1-phosphate, but recently, it was shown that, analogous to ATX, it can hydrolyze LPC to yield LPA, along with other lysophospholipids (42, 65). Strikingly, unlike ATX, which is autoinhibited by its products, LPA and S1P, sphingomyelinase D does not seem to be susceptible to autoinhibition. Because high concentrations of LPC are present in blood, sphingomyelinase D can induce prolonged and marked accumulation of lysophospholipids with pronounced pathologic effects. There are striking resemblances between LPA signaling and the noxious effects of envenomation: the production of interleukin-8 (68, 69) and growth-regulated oncogene α (69, 70), platelet aggregation (71), and endothelial permeability (65). Thus, cancer and the toxic effects of spider envenomation may represent a striking convergence. Because LPA receptors seem to play a role in the envenomation effects and because Loxosceles venom can produce LPA, LPA receptors should be considered a target for therapy after Loxosceles envenomation.

** Emerging therapeutics. ** The observation that ATX is autoinhibited suggested that stable LPA analogues, lacking receptor agonist properties, could potentially function as inhibitors of LPA production. A cyclized LPA analogue, cyclic phosphatidic acid (cPA), a bioactive lipid component of plasma (72), had previously been found to inhibit in vitro correlates of mitogenesis (73–75), invasion (76), and metastasis (43, 77), compatible with expected effects from the inhibition of LPA signaling. However, cPA did not act as an antagonist of LPA receptor signaling. In light of the ability of LPA to autoinhibit ATX, cPA and a series of carba analogues of cPA were found to inhibit ATX, reducing the pool of extracellular LPA, without activating LPA receptors (14). In vivo, cPA and its analogues decrease metastasis without exhibiting significant activity against primary tumors (14, 43). This not only indicates that the cPA analogues necessitate further preclinical development but also that LPA production and signaling represent novel targets warranting the development and implementation of high-throughput screening to identify additional inhibitors of ATX and LPA receptors. It will be intriguing to determine whether carba analogues of cPA will inhibit sphingomyelinase D, suggesting that they would also have therapeutic potential against Loxosceles envenomation.

Molecular therapeutics targeting S1P metabolism and signaling are closer to clinical evaluation than those for LPA. The
sphingosine analogue, FTY720, is under investigation in clinical trials to suppress organ rejection in kidney transplant patients and for the management of autoimmune diseases such as multiple sclerosis (34). Like sphingosine, this drug is phosphorylated by sphingosine kinase and is capable of binding all S1P receptors, except S1P2 (18). Interestingly, FTY720 has shown antitumor activity in preclinical models using multiple cell lineages both in vitro and in vivo (78–81). In addition, it has exhibited marked invasion inhibition and antimetastatic activity (79) as well as modest activity against local tumor growth, potentially by altering S1P receptor recycling in tumor cells (78). FTY720 may mediate its antimetastatic effects through inhibition of angiogenesis both indirectly through blocking production of angiogenic factors and directly by inhibiting the effects of S1P on viability, invasion, and maturation of tumor endothelial cells.

Because of their outstanding pharmacology, therapeutic antibodies including those that immunoneutralize molecules such as tumor necrosis factor α (enbrel) or vascular endothelial growth factor (bevacizumab) are highly attractive. In vivo testing showed that an anti-S1P immunoneutralizing monoclonal antibody (sphingomab) reduced tumor progression by inhibiting cell migration, S1P-mediated proliferation, neovascularization, and the release of proangiogenic cytokines vascular endothelial growth factor and IL8; suggesting that S1P is a validated target. As this antibody absorbs available S1P, the sphingolipid equilibrium is tipped towards ceramide and sphingosine production, two molecules that are proapoptotic and enhance the death pathway signaling in cells increasing the activity of sphingomab (21). Lpath, Inc. (San Diego, CA) has used the same approach to develop immunoneutralizing LPA antibodies providing the potential to neutralize both small lysophospholipids.

Without S1P receptor activation, vascular smooth muscle cells cannot form a layer around endothelial cells to establish a new blood vessel (73). Indeed, sphingomab inhibited the growth of tumors in vivo which were nonresponsive in vitro, indicating that the outcome of S1P immunoneutralization is mediated, at least in part, by an antivascular effect. Strikingly, sphingomab inhibited vessel formation induced by multiple factors including basic fibroblast growth factor, vascular endothelial growth factor, and S1P, implying that S1P may be the ultimate downstream mediator, or alternatively, permissive for neovascularization induced by multiple factors. Furthermore, this also suggests that sphingomab may be effective even in the face of plasticity in the utilization of neovascularization factors, potentially demonstrating activity in patients who have failed bevacizumab (21).

The best known in vitro inhibitor for sphingosine kinase is N,N-dimethylsphingosine, although this compound has off-target effects on PKC (45). Novel compounds that exhibit selectivity for Sphk2 have been identified recently; however,
more research is needed to determine whether they can be used in vivo (82). A recent report showed that, phenoxodiol, a synthetic analogue of genistein that inhibits sphingosine kinase, induces apoptosis in some cancer cells and is now in clinical trials (83). Sphingosine kinase is also sensitive to the effects of the chemotherapeutic drugs docetaxel and campto-

**Conclusions**

As the ligands, receptors, and molecular pathways that produce LPA and S1P and mediate their effects are emerging, therapeutic targets in cancer and an unusual variety of human ailments, novel targeted drugs will likely soon be available for therapeutic evaluation. The pleomorphic roles of lysophospholipids in cancer behavior suggest that they would have wide applicability across multiple tumor lineages. Thus, an understanding of the pathophysiologic functions of LPA and S1P in cancer will rapidly become important for clinicians and scientists if optimal translation to improved patient outcomes is to occur.

Unlike other targeted therapeutics, it is not yet possible to predict a molecular signature that will identify patients likely to respond to therapy. Thus, it may be necessary to evaluate a wide variety of cancers, obtaining tumor and blood to allow correlations with patient responses. The LPA and S1P antibodies described above may provide theragnostic tracking levels of the lysophospholipids in accessible body fluids.

A number of questions remain to be answered prior to determining the potential role of LPA and S1P as targets in cancer therapy. As S1P and LPA receptors are members of the G protein–coupled receptor family and over half of all drugs in clinical use are inhibitors of this family of receptors, the use of structure-function analysis or high-throughput screening to develop receptor inhibitors was thought to have great promise. However, it is not yet apparent which LPA or S1P receptors are the critical targets. For example, LPA3 has been reported to regulate motility and metastases to bone in breast models (15), but has also been reported to inhibit the growth of some cells when overexpressed (11). In contrast, LPA2 and LPA4 are frequently aberrant in cancer, suggesting that they may represent the critical receptors. Although LPA2 is strongly associated with the production of neovascularizing factors and LPA3 with cell survival, it seems that each of the receptors has the ability to mediate the major functions of LPA, with the relative efficiency being determined by the spectrum of receptors activated. Similarly S1P2 acts as an antagonist of the actions of other S1P receptors. Thus, pan receptor inhibitors of LPA and S1P may have unexpected consequences. Thus far, at least in animal models, each of the inhibitors has shown striking antitumor activity in the absence of apparent toxicity, suggesting the presence of a useful therapeutic window. Indeed, FTY720 has shown a reasonable therapeutic index in transplantation studies. However, given the differences in the physiology of rodents and humans, the realization of the true therapeutic index will require testing in human cancers and potentially other diseases. The rapid emergence of lead compounds warranting clinical evaluation suggests that new drugs will enter phase I trials and hopefully proceed through clinical testing to represent a new form of therapy. These studies will rapidly answer many of the questions above.

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