the presented studies do not test the self-renewal (serial transfer of neurospheres) or potency of glioma cells that are more rigorous analyses of the stem cell fraction. Studies of glioma cells isolated from fresh samples of *PN*, *Prolif*, and *Mes*, signature tumors using more formal tests of stem cell function would better determine whether these expression profiles report the stem cell phenotype of HGG. These studies might also help to determine if the different expression subtypes of HGG are maintained by a common cell type, or represent more distinct forms of the disease that arise from transformed cells at different stages along the neural differentiation pathway. Interestingly, the authors demonstrate that some tumors display a switch in expression signature from *PN* to *Mes* following disease progression (Figure 1). Thus, expression subtypes of HGG may not be entirely distinct, but rather represent different stages or forms of a more common disease process. Comparison of the *PN*, *Prolif*, and *Mes* signatures to those of the available mouse models of glioma that have been derived from cells in different stages of differentiation (Bachoo et al., 2002) may provide further clues to the cellular origins of these HGG subtypes. Finally, the findings of Phillips et al. also have implications for the development of novel therapies of HGG. Perhaps the most significant of these is the demonstration that tumors with *PN* and *Prolif*/*Mes* signatures display evidence of activation of the NOTCH and AKT cell signal pathways, respectively. These cell signaling systems have been identified previously as potential targets for glioma treatment. Therefore, the efficiency of clinical trials that test inhibitors of NOTCH or AKT signaling could be increased significantly by enrolling patients whose tumors display the *PN* or *Prolif*/Med expression signature, respectively.

The extensive expression profiling analysis by Phillips et al. represents an important step forward in our understanding of the biology and treatment of HGG. Their integrated approach has provided important clues that may allow us ultimately to identify the distinct molecular processes that result in the long-recognized clinical and pathologic forms of these devastating diseases.

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Targeting sphingosine-1-phosphate: A novel avenue for cancer therapeutics

Sphingosine-1-phosphate (S1P) is a pleiotropic lipid mediator that has been shown to regulate cell growth, cell survival, cell invasion, vascular maturation, and angiogenesis, processes that are important for cancer progression. In this issue of *Cancer Cell*, Visentin et al. demonstrate that a monoclonal antibody that binds S1P with extremely high affinity and specificity significantly slows tumor progression and associated angiogenesis in several animal models of human cancer. Their results suggest that S1P not only affects tumor cells themselves, but also is permissive or required for the actions of angiogenic factors, and thus may be a bona fide cancer target.

Sphingosine-1-phosphate (S1P) is the simplest and most intriguing sphingolipid metabolite. Although S1P was initially considered as an intermediate in the ultimate degradation of all sphingolipids, its bewildering nature is rapidly being deciphered, and it is now emerging as a vital lipid mediator of a myriad of cellular processes important for cancer. S1P exerts most of its actions as a specific ligand for a family of five cognate G protein-coupled receptors, designated $S1P_{1-5}$, which regulate cytoskeletal rearrangements and cell movement, angiogenesis and vascular maturation, and immunity and lymphocyte trafficking. This potent lipid may also have intracellular func-

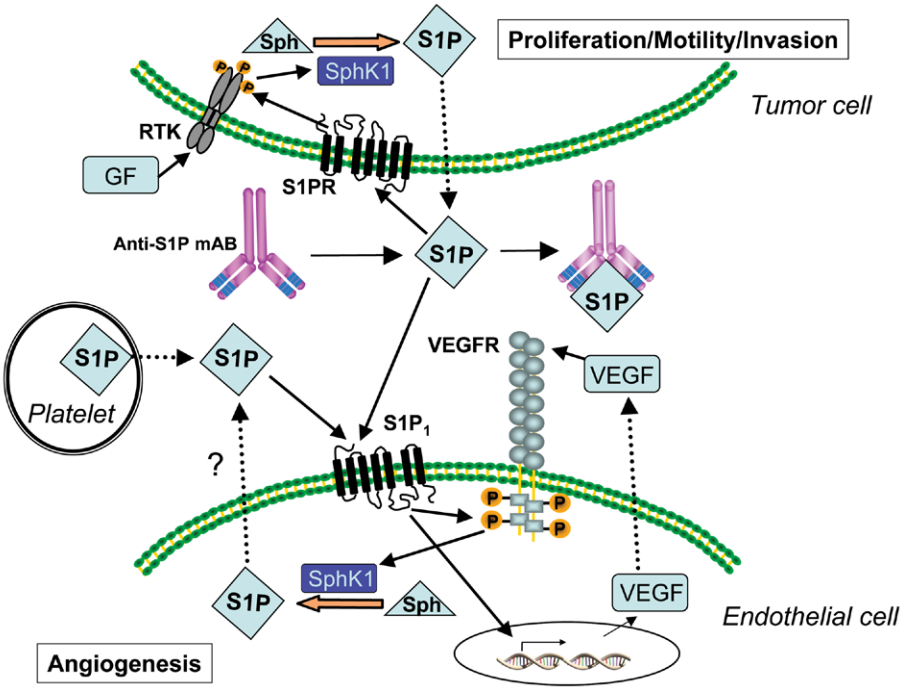

Figure 1. Crosstalk between S1P receptors and growth and proangiogenic factor receptors can lead to the amplification of signals important for tumorigenicity and angiogenesis

In addition to platelets that secrete S1P, growth factors (GF) activate RTKs, causing upregulation and stimulation of SphK1 and production of S1P that can be released from tumor cells by uncharacterized mechanisms. This S1P in turn can act in an autocrine or paracrine manner to activate S1PRs present on the tumor cell or on endothelial cells and stimulate canonical signaling downstream of heterotrimeric G proteins and can also transactivate RTKs. This leads to signal amplification loops that regulate tumor growth, survival, invasion, vascular remodeling, and angiogenesis. It is also possible that transactivation of S1PRs and RTKs is facilitated by their colocalization mediated by a scaffolding protein in a signaling complex (signalplex). Thus, a mAb that neutralizes S1P could slow tumor progression and angiogenesis by interfering at multiple sites of S1P actions. For simplicity, known signaling pathways downstream of S1PRs or RTKs are not shown.

tions important for calcium homeostasis, cell growth, and suppression of apoptosis (Spiegel and Milstien, 2003). In contrast to prosurvival S1P, its precursors, sphingosine and ceramide (N-acyl sphingosine), have been associated with growth arrest and apoptosis. Because these metabolites are interconvertible, their dynamic balance, the so-called "sphingolipid rheostat," and their regulation of opposing signals can determine whether cells live or die. Thus, it is not surprising that increased S1P or attenuated sphingosine and/or ceramide levels have been implicated in various stages of cancer pathogenesis, including antiapoptotic phenotype, metastasis, escape from senescence, and resistance to chemotherapy and radiation therapy.

S1P levels in cells are low and tightly regulated both spatially and temporally. S1P is produced by phosphorylation of sphingosine by two sphingosine kinase isoenzymes (SphK1 and SphK2) and can be dephosphorylated by several phosphatases or cleaved irreversibly by a lyase. SphK1 may be oncogenic, since it enhances cell growth and colony formation in soft agar, confers resistance to radiation and chemotherapy, and promotes tumorigenesis in nude mice. SphK1 is elevated in a variety of solid tumors, and inhibitors of SphK1 reduce gastric and mammary adenocarcinoma tumor growth in mice (French et al., 2003). One such inhibitor is now in clinical trials in patients with advanced solid tumors in combination with cisplatin.

Importantly, many growth and proangiogenic factors that have been implicated in cancer progression, including EGF, PDGF, and VEGF, stimulate and translocate SphK1 to the plasma membrane, resulting in local formation of S1P (Pitson et al., 2005) and activation of S1P receptors (S1PRs) (Spiegel and Milstien, 2003), such as $S1P_1$, which plays a critical role in angiogenesis and vascular maturation (Liu et al., 2000). Binding of S1P to the $S1P_1$ receptor can also increase PDGF and VEGF production, leading to transactivation of the growth factor receptors that in turn activate downstream signals that regulate vascular remodeling and cell movement. Despite these observations, until now there was no direct definitive evidence that S1P plays a critical role in tumor growth, metastases, and angiogenesis. In this issue of *Cancer Cell*, Visentin et al. (2006) show that neutralizing S1P with a specific monoclonal antibody was remarkably effective in retarding progression of deadly and multiresistant cancers such as lung, breast, melanoma, and ovarian cancers in murine xenograft and allograft models. Their studies suggest that the principal mechanism of the anti-S1P mAb is prevention of the proangiogenic effects of the blood-borne lipid mediator S1P.

As there are so many proangiogenic factors, including VEGF, bFGF, angiopoietin-1, IL-6, IL-8, and PDGF, among others, a critical question is how neutralization of this simple lysophospholipid can have such dramatic effects on tumor angiogenesis. Some answers are provided by Visentin et al. (2006), as this S1P "sponge" not only blocked the functions of VEGF and bFGF in vivo, but also inhibited the release of VEGF, IL-6, and IL-8 in vivo and in vitro. Thus, either S1P is the central and obligatory mediator of the actions of proangiogenic factors or its signaling is permissive for the angiogenic effects.

Complex crosstalk between S1P and growth and proangiogenic factor signaling pathways might explain these results (Figure 1). S1P by binding to $S1P_1$ can transactivate growth factor receptor tyrosine kinases (RTK), such as VEGFR, EGFR, and PDGFR, through three mechanisms that are not mutually exclusive: (1) intracellular receptor crosstalk and direct phosphorylation of the RTK by protein tyrosine kinases; (2) induced production and/or secretion of the growth factors; and (3) by participation of $S1P_1$ and RTK in a signalplex, either by direct receptor/receptor interaction or by binding of both receptors to

scaffolding protein. Conversely, binding of these growth factors to their RTKs can also translocate and activate SphK1 (Pitson et al., 2005), leading to spatially restricted formation of S1P that in turn further activates canonical S1PR signaling ("inside-out signaling") (Hobson et al., 2001). In addition, these RTKs can also upregulate $S1P_1$ (Igarashi et al., 2003). Thus, S1P might be the central controller of several amplification loops. Moreover, interplay between the components of a signalplex that is necessary for tumor-driven angiogenesis may be much more intricate than linear transactivation of one receptor by another. This paradigm fits with the emerging view of the complexity and nonlinearity of signaling via S1PRs and RTKs, and of the importance of membrane compartmentalization to signal transduction (Neve, 2005).

Another important question raised by the work of Sabbadini and colleagues is as follows: what is the source of active S1P that is being neutralized by their mAb? Serum contains significant amounts of S1P, probably mainly arising by release from abundant platelet stores (Yatomi et al., 1995). However, most of this S1P is tightly bound to HDL and serum albumin, which protects it from degradation and decreases its bioavailability. Sabbadini and colleagues calculated that the dose of S1P mAb administered to mice that effectively blocked tumor growth reached blood levels that were about 3-fold higher than S1P levels on a molar basis. The free concentration of S1P is determined by equilibria between formation, elimination, binding, and compartmentalization. It is possible that "free" S1P in serum is permissive for angiogenesis, and neutralizing it would block this necessary S1PR signaling. A less likely alternative is that the S1P-mAb complex acts as an antagonist to block vital S1PR functions. Finally, a most attractive possibility is that it is S1P produced and secreted by the tumors themselves or by stromal cells or other cells attracted to the tumor that mediates the growth of tumor-feeding blood vessels. In support of this notion, SphK1 is upregulated in many tumor cells (French et al., 2003), and $S1P_1$ receptor expression is also induced in angiogenic vessels in vivo. However, it is still a mystery how the polar lipid S1P produced in cells by activation of SphK1 can cross the lipid bilayer and reach receptors on the surface of cells. If the anti-S1P mAb effectively sucks S1P from cells, it might have the added advantage of decreasing the intracellular ratio between S1P and its precursors, sphingosine and ceramide, thus favoring cell growth arrest and apoptosis. In agreement, recent studies suggest that cytosolic S1P formed by SphK1 inhibits ceramide biosynthesis, possibly as a cellular sensing mechanism to regulate levels of ceramide, and downregulation of SphK1 enhances ceramide levels and apoptosis (Maceyka et al., 2005). This paper by Visentin et al. thus provides proof of concept that direct targeting of important lipid signaling molecules is a novel strategy for development of new types of cancer treatments that could be useful in combination with other treatment strategies.

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