Given the existence of multiple Smo-independent mechanisms of Hh pathway activation, such as gene amplification or increased activity of the Gli transcription factors as well as inactivation of the intracellular repressor Sufu, inhibition of Gli transcriptional effectors appears appealing. Of relevance, activation of the S6K1 kinase in the PI3K/mTOR pathway has recently been shown to increase active GLI1 levels (Wang et al., 2012), which may contribute to the observed Smo inhibitor resistance caused by increased PI3K/mTOR signaling.

A concern with regard to the clinical use of Smo inhibitors is side-effects such as weight loss and muscle cramping that have no known connection to canonical Hh-signaling. Smo-dependent activation of AMPK and Ca^{2+} influx in brown fat and muscle cells elicited by several Smo inhibitors is now proposed as an underlying mechanism (Teperino et al., 2012). Investigation of the presence or absence of such activity induced by itraconazole is therefore warranted.

The findings by Kim et al. (2013) are, despite unanswered questions, very encouraging in that they suggest immediately available second-line treatment options for Smo inhibitor-resistant tumors to be evaluated in the clinical setting.

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Sphingosine 1-Phosphate Is a Missing Link between Chronic Inflammation and Colon Cancer

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In this issue of *Cancer Cell*, Liang and colleagues demonstrated that sphingosine kinase 1, the enzyme that catalyzes formation of the biologically active lipid sphingosine 1-phosphate, drives a malicious amplification loop involving sphingosine 1-phosphate receptor 1 and the NF-κB/IL-6/STAT3 pathway. This appears critical for progression from chronic inflammation to colon cancer.

There is substantial evidence linking inflammatory bowel disease, particularly ulcerative colitis (UC), with colon cancer, termed colitis-associated cancer (CAC). In this regard, mucosal inflammatory cell types can promote (regulatory T cells [Tregs], type 2 macrophages, $CD4^+$ T helper [Th-17 cells]) or inhibit ($CD8^+$ T cells, natural killer cells) CAC (Monteleone et al., 2012). The regulatory effects of inflammatory cells on the growth and survival of cancer cells is dependent on cytokines that can act directly or indirectly on these cells. For instance, cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) provide a supportive tumor microenvironment by activating the transcription factors NF-κB and STAT3, and these are involved in the development of CAC. Activation of NF-κB in a tumor microenvironment is associated with enhanced inflammation and release of IL-6, which promotes CAC (Monteleone et al., 2012).

In this issue of *Cancer Cell*, Liang et al. (2013) elegantly demonstrate that the sphingosine 1-phosphate receptor 1 (S1PR1) and sphingosine kinase 1 (SphK1), the enzyme that catalyzes the formation of sphingosine 1-phosphate (S1P, the natural ligand of S1PR1), are a missing link between chronic inflammation and CAC. Indeed, there is substantial evidence for a role of SphK1 and S1P in cancer (Pyne and Pyne, 2010). There were two important findings that preceded the current study. First, Kawamori et al. (2009) demonstrated that *Sphk1* knockout mice exhibit reduced

aberrant crypt foci formation and tumor development in a CAC model. Second, Lee et al. (2010) demonstrated that STAT3 induces the upregulation of S1PR1, which reciprocally activates STAT3, resulting in persistent IL-6 formation to drive tumor growth and metastasis in B16 melanoma and MB49 bladder cancer models. The advance made by Liang et al. (2013) is the demonstration that the NF-κB/IL-6/STAT3/S1PR1 amplification loop is functional in both colitis and CAC adenomas and is driven by SphK1, which is upregulated in UC (Snider et al., 2009). Moreover, Liang et al. (2013) were able to ablate this amplification loop in both colitis and CAC using the SphK1 inhibitor FTY720.

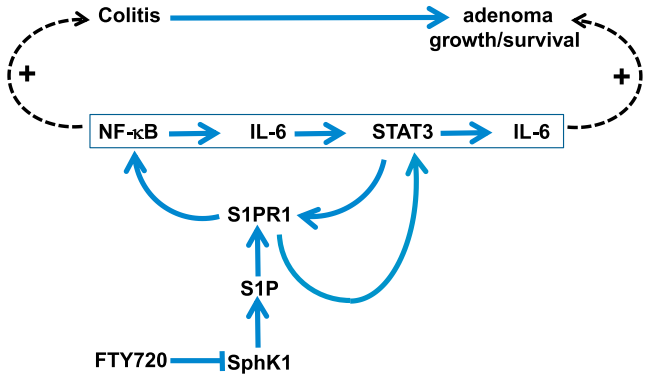

Figure 1. Schematic Showing how SphK1 and S1P Promote the NF-κB/IL-6/STAT3/S1PR1 Amplification Loop that Drives Colitis and CAC

FTY720 ablates colitis and CAC through inhibition and downregulation of SphK1. The S1P → S1PR1 → NF-κB connection is represented as W146 ablates S1P-stimulated NF-κB activation in naive *Sphk2*$^{-/-}$ mice. The possible S1P → S1PR1 → STAT3 → IL-6 connection is represented as Lee et al. (2010) demonstrated that overexpressing S1PR1 increased IL-6 formation in MB49 bladder cancer cells, and this was eliminated in *Stat3*$^{-/-}$ mice.

Liang et al. (2013) found that when mice deficient of SphK2 (the second SphK isoform) were injected with colontropic mutagen azooxymethane followed by oral administration of dextran sodium sulfate (DSS), there was a marked increase in tumor number, size, load, and Ki-67 staining compared with wild-type (WT) mice. Indeed, there was a similar increase in intestinal inflammation in response to DSS compared with WT mice. Significantly, deletion of *Sphk2* was associated with a marked increase in SphK1 expression, which was shown to be due to the loss of a SphK2-mediated inhibition of HDAC1/2, leading to increased expression of c-Jun, a component of AP-1 that regulates SphK1 expression. The authors therefore concluded that the enhancing effect of *Sphk2* knockout on colitis and CAC might be due to the increase in expression of SphK1. Constitutive NF-κB activation, nuclear phosphorylated STAT3, elevated S1PR1 expression, and IL-6/TNF-α formation were increased in *Sphk2*$^{-/-}$ mice and further enhanced during colitis development. The role of "inside-out" signaling involving SphK1/S1P/S1PR1 in colitis was confirmed using either the SphK1 inhibitor BML-258 (SK1-I) or a competitive antagonist of S1PR1, W146, indicating a requirement for S1P formation and subsequent activation of S1PR1 rather than constitutive activation of this receptor. Similarly, the amplification loop appears operative in naive *Sphk2*$^{-/-}$ mice where W146 reduced activation of NF-κB and STAT3.

Importantly, reciprocal bone marrow chimeric mice, generated by the adoptive transfer of bone marrow into lethally irradiated recipients, were used to demonstrate that hematopoietic cells rather than intestinal epithelial cells (IECs) drive NF-κB/IL-6 and STAT3 activation and colitis and that the principal cells involved are macrophages, probably of the M1 phenotype. Colitis is then directly linked with CAC, with the demonstration that late phase CAC involves infiltration of macrophages and T cells in the adenoma leukocyte infiltrates of DSS-challenged *Sphk2*$^{-/-}$ mice.

FTY720 has been marketed for the treatment of multiple sclerosis (MS) and is a functional antagonist of S1PR1 (Brinkmann et al., 2010). FTY720 is converted to FTY720 phosphate by SphK2, which subsequently binds to S1PR1 on T cells to induce its downregulation, thereby preventing egress of T cells from lymph nodes. This mechanism of action of FTY720 phosphate is thought to underlie its therapeutic utility in MS. Liang et al. (2013) demonstrated that daily treatment with FTY720 reduced colitis in WT and *Sphk2*$^{-/-}$ mice. Moreover, FTY720 induced lymphopenia in *Sphk2*$^{-/-}$ mice, and it was proposed that FTY720 can be phosphorylated by SphK1 albeit as a poor substrate. Non-phosphorylated FTY720 is a competitive inhibitor (with sphingosine) of SphK1 (Tonelli et al., 2010), and its binding site on SphK1 probably overlaps the catalytic site of SphK1 so that it might serve as a substrate while inhibiting phosphorylation of sphingosine.

FTY720 also has anti-cancer activity in vivo (Pyne and Pyne, 2010) and inhibits the S1PR1 activation of STAT3 in activated B cell-like diffuse large B cell lymphoma (Liu et al., 2012). Moreover, non-phosphorylated FTY720 induces the ubiquitin-proteasomal degradation of SphK1 (Tonelli et al., 2010). Indeed, the proteasomal degradation of SphK1 might explain how FTY720 reduces the increase in SphK1 expression in the inflamed colon of WT mice induced by DSS treatment, as well as dramatically decreasing SphK1 expression in *Sphk2*$^{-/-}$ mice. This effect of FTY720 is likely to be temporally separated from its use as a substrate by SphK1 and as a catalytic inhibitor of S1P formation. Liang et al. (2013) also demonstrated that FTY720 abrogated the SphK1/S1P/S1PR1 feed-forward loop that leads to persistent NF-κB and STAT3 activation, IL-6 production, and macrophage recruitment in colitis. In addition, FTY720 reduced tumor size, multiplicity, and load in WT and *Sphk2*$^{-/-}$ mice, and administration at late stage CAC induction was effective in reducing the proliferation rate in WT mice. This was also associated with a reduced level of nuclear phosphorylated STAT3, which regulates proliferation and IL-6 production, while NF-κB activation was reduced in IECs of CAC adenomas from *Sphk2*$^{-/-}$ mice. Moreover, late FTY720 administration reduced elevated SphK1 and S1PR1 expression in CAC adenomas and in distal IECs of the *Sphk2*$^{-/-}$ mice. Therefore, FTY720 was effective in ablating activation of NF-κB and STAT3 and reducing IL-6 levels even in established tumors.

The link between SphK1/S1P/S1PR1 and NF-κB/IL-6 and STAT3 activation

might represent the Achilles heel of CAC. Therefore, targeting this amplification loop with FTY720 or FTY720-based SphK1 inhibitors might offer the prospect of being able to halt progression of colitis to colon cancer. There is a strong possibility that SphK1 provides a proliferative and survival advantage to CAC cells that enables their clonal expansion through activation of STAT3 and NF-κB. In addition, Deng et al. (2012) have shown that the S1PR1/STAT3 amplification loop leads to pre-metastatic niche formation, which has important implications in terms of targeting SphK1 and S1PR1 to prevent metastasis. Therefore, understanding the molecular mechanisms for the deregulation of SphK1 in colitis and CAC seems a pre-eminent consideration for future research.

The current findings presented by Liang et al. (2013) represent a major advance in the area of CAC. Significantly, the identification of the NF-κB/IL-6/STAT3/S1PR1 amplification loop driven by SphK1 (Figure 1) provides a viable target for intervention in colitis to prevent transition to CAC. This might represent a particularly innovative approach as chemotherapeutic resistance is less problematic in inflammatory cells compared with tumors.

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