Sphingosine Kinase 2 Promotes Acute Lymphoblastic Leukemia by Enhancing MYC Expression

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Abstract

Sphingosine kinase 2 (SK2) may have utility as a prognostic marker in inflammatory diseases such as cancer in which it has been rationalized as a candidate therapeutic target. Here, we show that SK2 has an oncogenic role in acute lymphoblastic leukemia (ALL) by influencing expression of MYC. Genetic ablation of SK2 impaired leukemia development in a mouse model of ALL and pharmacologic inhibition extended survival in mouse xenograft models of human disease. SK2 attenuation in both the settings reduced MYC expression in leukemic cells, with reduced levels of acetylated histone H3 within the MYC gene associated with reduced levels of MYC protein and expression of MYC-regulated genes. Our results demonstrated that SK2 regulates MYC, which has a pivotal role in hematologic malignancies, providing a preclinical proof of concept for this pathway as a broad-based therapeutic target in this setting. Cancer Res; 74(10); 1–13.

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Introduction

Sphingosine kinases (SK) 1 and 2 catalyze the synthesis of sphingosine 1-phosphate (SIP) from sphingosine (1). Overexpression of SK1 has indisputable tumor-promoting properties (2) and has been associated with poor survival in patients with solid tumor (3–5), resistance to imatinib in chronic myeloid leukemia (CML; refs. 6–8), and to daunorubicin in acute myeloid leukemia cells (9). In contrast, the role of SK2 in cancer is less clear. While overexpression suppressed cell growth and induced apoptosis (1), knockdown of SPHK2 in glioblastoma cells inhibited proliferation more effectively than knockdown of SPHK1, and SPHK2-deficient breast cancer cells proliferated poorly in vitro (5, 10, 11). SK2 has basal activity that is enhanced by extracellular signal-regulated kinase (ERK)–mediated phosphorylation (12), placing it downstream of the signaling pathways frequently activated in malignancies, including B-Raf, Ras, Jak, and Flt3 (13, 14), all of which can activate ERK (15).

SIP is best known for its extracellular effects mediated through G protein–coupled receptors. SIP1 to SIP5. SIP can also act intracellularly by direct modulation of histone deacetylase (HDAC) activity and the ubiquitin ligase activity of tumor necrosis factor receptor–associated factor 2 (TRAF2; ref. 1). In the nucleus, SK2 binds directly to HDACs where locally produced SIP inhibits HDAC1 and 2, increasing acetylation of histone H3 on Lys9, H4 on Lys8, and H2B on Lys12 (16). Gene expression of the cell-cycle inhibitor p21 and transcriptional regulator c-fos is subsequently increased, providing an explanation for the reported growth-inhibitory effects of SK2.

The Myc proto-oncogene protein (c-Myc) is overexpressed across a diverse range of human cancers (17), including hematologic malignancies, playing a role in both tumor initiation and maintenance (18). In acute lymphoblastic leukemia (ALL), the role of c-Myc has not been extensively explored; however, translocations involving MYC have been reported in some cases (19, 20). Normally MYC expression is tightly controlled by transcriptional regulatory motifs in its proximal promoter region (21); however, epigenetic modifications can also influence the expression of MYC, including in ALL (22).

We demonstrate that a gene signature consistent with increased SK2 activity is present in ALL patient diagnostic samples and that SK2 protein is overexpressed and activated in ALL cells. Inhibition of SK2 kills B-lineage ALL cells and synergizes with the proteasome inhibitor bortezomib and with imatinib in Philadelphia chromosome–positive (Ph+) cells in vitro. SK2 inhibition reduced expression of MYC mRNA, protein, and c-Myc target genes. Reduced association of acetylated histone H3 with MYC was observed, a finding consistent with the effect being mediated by the role of SK2 in histone acetylation. In vivo, SK2 inhibition showed significant antileukemic effects in a human xenograft model of ALL, and enhanced the efficacy of imatinib, resulting in increased...
survival in mice bearing a Ph⁺ xenograft. Furthermore, deletion of Sphk2 significantly reduced leukemia development in a murine model of BCR/ABL–driven ALL.

Materials and Methods

Cells

Cell lines used were ALL1 (Dr. J. Radich, Fred Hutchinson Cancer Research Center, Seattle, OR), 2070 (23), TOM1 (Prof. J. Melo, IMVS, Adelaide, South Australia), REH, K562 (both from American Type Culture Collection), NALM6 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH), LK63 (Prof. A. Boyd, QIMR, Brisbane, Queensland), and stromal-dependent ALL lines derived in our laboratory from patient samples (2032, 1345, 2070, and 1809). Leukemic blasts were obtained from patients with ALL with informed consent and institutional ethics committee approval from the Sydney West Area Health Service Human Research Ethics Committee. Spleen cells from previously xenografted animals were used in the in vivo experiments except sample 2070, in which cryopreserved patient cells were used. Patient details are in Supplementary Table S1 and previous articles (23, 24).

Antibodies and reagents

ABC294640 was obtained from Enzo Life Sciences, the SK1 inhibitor PF543 was obtained from Merck Millipore, the c-Myc inhibitor, 10058-F4, and 3-methyladenine (3MA) from Sigma-Aldrich, bortezomib from Selleck Chemicals, and Z-VAD-FMK from BD Biosciences. The specificity of ABC294640 and PF543 was determined in isoform specific assays (Supplementary Fig. S1). Antibodies to the following were purchased: cleaved caspase-3-FITC, CD19-PE, mCD45R/B220-PE-Cy5, mCD19-PE, mCD11b-FITC (BD Biosciences), mCD45-FITC (Invitrogen), LC3, histone H3 acetylated on Lys9, c-Myc from Cell Signaling Technology, and SK2, phospho(Thr²⁷⁸)-SK2 from ECM Biosciences.

Gene expression analysis

Total RNA extracted from cells using the Qiagen RNeasy Mini Kit was amplified and biotinylated with the TargetAmp-Nano Labeling Kit (Epicentre Biotechnologies), and gene expression analyzed using the illumina HumanHT-12 v4.0 Whole-Genome Gene Expression BeadChip and GenomeStudio software.
Generation of gene signatures

Gene signatures were generated using normalized data (GenomeStudio) from cell lines treated with 60 μmol/L (ALL1, TOM1, and NALM6) or 80 μmol/L (REH) ABC294640 for 24 hours. Data were filtered using a differential expression (Diff) P value of < 0.01 or 2-fold regulation followed by principal component analysis (Qlucore; P < 0.005; false discovery rate < 0.01). The validation cohort was generated using 80 μmol/L ABC294640-treated 2032, 1345, 2070, 1809, and LK63 cells. Hierarchical clustering was performed using the GenePattern website and the Pearson or Spearman correlation for the column distance measure and Pairwise complete-linkage method, on data normalized for each row and centered on the mean. Heat maps were generated using Java Treeview. The signal intensity for genes was extracted from GSE28497. The mean of the log2-transformed data of the upregulated genes was calculated and the signature intensity was expressed as the change in this value in patient samples relative to that in the normal controls.

Flow cytometry, viability, and proliferation assays

Viability was measured using propidium iodide (PI) and Annexin V–FITC (BD Biosciences) staining and proliferation was measured by 3H-thymidine incorporation both as previously described (25). Intracellular caspase-3 staining was performed as previously described (26), using FACSCanto or LSRFortessa flow cytometers (BD Biosciences).

Assessment of drug interactions

Cells were cultured alone or with IC50 concentrations of agents to be tested when used singly and viability was assessed by flow cytometry. The proportion of surviving cells expected if no interactive effects were observed was calculated according to the method of Webb (27).

Chromatin immunoprecipitation assay

Protein was cross-linked to DNA for 10 minutes at 37°C with 1% formaldehyde and then quenched with 125 mmol/L glycine. Cells were lysed in 1% SDS, 10 mmol/L EDTA, 50 mmol/L Tris-HCl, pH 8.0 for 10 minutes on ice and sonicated using five 1-minute cycles at 40% amplitude with 60% duty from a Branson sonicator. Lysates were clarified by centrifugation at 10,000 × g for 10 minutes at 4°C and diluted 10-fold in IP buffer [0.5% NP-40; 50 mmol/L Tris, pH 8; 120 mmol/L NaCl; 0.5 mmol/L PMSF; 0.5 mmol/L EDTA; 1 mmol/L NaF; 1 mmol/L Na3VO4].

SIP quantitation and Western blotting

SIP concentrations in mouse plasma and cell lysates were determined using an SIP ELISA Kit (Echelon Biosciences Inc.) according to the manufacturer’s instructions. Western blotting was performed as previously described (24).
complete protease inhibitor cocktail (Roche)]. Lysates were precleared for 1 hour at 4°C with protein G magnetic beads (Invitrogen) and then incubated with primary antibody and protein G magnetic beads overnight at 4°C. Beads were sequentially washed with IP buffer, high-salt wash buffer (IP buffer containing 500 mmol/L NaCl), lithium wash buffer (250 mmol/L LiCl; 0.5% NP-40; 1% sodium deoxycholate; 1 mmol/L EDTA, and 10 mmol/L Tris-HCl, pH 8.0), and Tris/EDTA (10 mmol/L Tris and 1 mmol/L EDTA, pH 8.0). The protein–DNA complexes were eluted with 1% SDS in 0.1 mol/L NaHCO₃ at room temperature for 15 minutes, cross-links were reversed in 50 mmol/L NaCl at 65°C for 6 hours, and DNA was purified using QIAquick PCR columns (Qiagen).

**PCR, quantitative PCR, and quantitative real-time PCR**

**BCR/ABL** was amplified from genomic DNA using Takara Taq HS (Takara Bio Inc.) and primers specific for the e1a2 transcript of BCR/ABL and Sphk2 using primers (see Supplementary Data). Products were separated on agarose gels and stained with ethidium bromide (Amresco; 10 µg/mL) and visualized using Molecular Imager Gel Doc XR (Bio-Rad).

RNA was extracted, reverse transcribed, and quantitative RT-PCR performed using SYBR Green Real-Time PCR Master Mix (Invitrogen). Products were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and analyzed on Rotor-Gene software. Further details are in Supplementary Data and Supplementary Table S2.

**Leukemia models**

Mice were used with permission from the Westmead Hospital Animal Ethics committee and experiments were performed under guidelines from the Australian code of practice for the care and use of animals for scientific purposes 7th Edition, 2004.

**Induction of BCR/ABL–positive ALL in mice.** pMSCV retroviral vectors containing a 5′-LTR–driven p185 BCR/ABL and internal ribosome entry site (IRES) enhanced GFP (provided by Dr. C. Mulligan, St Jude Children’s Research Hospital, Memphis, TN) were used to generate helper-free retrovirus complexes. Retroviral particles were produced and a log-rank test used to determine significance of differences as required due to deteriorating health scores.

**Assessment of in vivo ABC294640 efficacy.** NOD.Cg-Prkdcsd12Rbgtm1Wjl/Sjij (NOD/SCIDγc–/–) mice were given 2 to 5 × 10⁶ ALL cells by tail-vein injection. Three (xenografts 1999 and 2070) or 7 (xenografts 1999 and 2070) days post injection of leukemic cells, 50 mg/kg (xenograft 2070), 100 mg/kg ABC294640 (xenografts 1345 and 1999), or vehicle (PBS) were administered daily for 21 days by intraperitoneal injection and the animals were sacrificed. The percentage of human cells in the peripheral blood (assessed weekly), BM, and spleen was assessed by flow cytometry as described (28). Total leukemia was calculated on the basis of the BM from one femur representing 5.8% of the total BM and a blood volume of 80 mL/kg of body weight.

For survival studies, mice engrafted with ALL as above were treated for 21 days with 100 mg/kg of ABC294640 when approximately 1% leukemia was detected in the blood. Animals were sacrificed as required due to deteriorating health scores.

**Statistical analysis**

The expression of the gene signatures in ALL cells was performed using the Mann–Whitney test. The fractional product method (27) was used to discriminate between synergistic and antagonistic effects of drug combinations with a value of >0.1 indicating antagonism and <−0.1 indicating synergy. Comparisons between two groups were performed using the Student t test and between multiple groups using ANOVA analysis. A level of significance of < 0.05 was deemed significant. For survival studies, Kaplan–Meier analysis was performed and a log-rank test used to determine significance with Bonferroni’s correction for multiple comparisons.

**Results**

**Pharmacologic inhibition of SK2 reduces ALL cell proliferation and survival in vitro and induces caspase-independent cell death**

Here, we show that ABC294640 reduced SK2 activity and S1P production in leukemic cell lines but did not affect SK1 activity or protein expression (Fig. 1A and Supplementary Figs. S1 and S4A). SK2 inhibition consistently reduced cell proliferation (Fig. 1B and data not shown), and induced cell death (Fig. 1C) in a time-dependent manner (Fig. 1E) with IC₅₀ values at 24 hours being significantly higher than those measured at later time points (P < 0.001, ANOVA test with repeated measures). Furthermore, SK2 inhibition also induced cell death in patient ALL cells (Fig. 1D) while IC₅₀ concentrations for normal BM mononuclear cells and B-cell progenitors were not achieved even after 72 hours (Supplementary Fig. S5).

Inhibition of SK2 resulted in externalization of phosphatidylserine (Fig. 1F); however, a transition from Annexin V+/PI– (early apoptosis) to Annexin V+/PI+ (late apoptosis) was not clear (Fig. 1F). Apoptotic cells, as defined by nuclear condensation and apoptotic bodies on examination by light microscopy, were largely absent in ABC294640-treated cultures (Supplementary Fig. S6) despite cleavage of caspase-3 (Fig. 1G). Although caspase inhibition significantly inhibited apoptosis induced by doxorubicin, it did not significantly reduce ABC294640-induced cell death (P = 0.18; n = 8; Fig. 1H). Although LC3-I was processed to LC3-II following SK2 inhibition (Fig. 1I), the autophagy inhibitor 3MA did not diminish ABC294640-induced cell death (Fig. 1J), despite...
reducing LC3 processing (data not shown). Overall, cell death following SK2 inhibition was largely caspase-independent and lacked morphologic features of apoptosis. Although autophagy was occurring following ABC294640 treatment, this was not the mechanism of cell death.

SK expression in ALL

To establish a gene signature for activation of SK2, we treated ALL cell lines with the SK2-specific inhibitor ABC294640 and analyzed gene expression by microarray. A Diff P value filter produced a list of 12 probes representing 11 genes, and principal component analysis identified 40 probes representing 35 genes (Fig. 2A). Notably, a gene signature could not be generated from cells treated with the SK1-specific inhibitor SK1-I (29) and the genes regulated by the SK2 inhibitor were not altered in cells treated with the SK1 inhibitor SK1-I (Supplementary Fig. S7), demonstrating specificity of the signature to SK2. Regulation of selected genes was confirmed by quantitative real time (qRT-PCR; Supplementary Fig. S8). Both SK2 gene signatures segregated control and ABC294640-treated cell lines using hierarchical clustering and Qlucore software (Fig. 2A and data not shown) and were validated using an additional five cell lines (Supplementary Fig. S9). In contrast, these signatures could not separate cells treated with SK1-I from control-treated cells (Supplementary Fig. S7). The SK2 signatures were used to interrogate a large publicly available gene expression dataset obtained from pediatric patients with ALL at the time of diagnosis (GSE28497; ref. 30). SK2 activity signatures were higher in ALL samples (P = 0.001 and P = 0.027; Fig. 2B and data not shown) than normal B-cell progenitors. The SPHK2 gene was not overexpressed; however, increased SK2 protein was detected (Fig. 2C) with increased phosphorylation, suggesting increased enzymatic activity (Fig. 2C). Together, these data show activation of SK2 without increased gene expression.

SK2 inhibition in ALL inhibits expression of MYC and c-Myc–regulated genes

To determine the mechanism by which inhibition of SK2 induced cell death in ALL, we undertook microarray analysis of control and ABC294640-treated cells at the earlier time...
point of 6 hours. Only five genes were consistently regulated across all cell lines (Fig. 3A), and these genes were not consistently regulated in SK1-I-treated cells (data not shown). Examination of the diagnostic pediatric ALL gene expression dataset, GSE28497 (30), revealed MYC to be more than 2-fold overexpressed ($P = 0.03$), affecting all subclassifications (Fig. 3B). FAM129A was slightly overexpressed ($\log_2$ fold change, 0.84; $P = 0.0001$) and DDIT3 was underexpressed.
SK2 inhibition reduces the association of histone H3 acetylated on lysine 9 with the MYC promoter

It has been reported that SK2 binds to HDAC in the nucleus and that locally produced S1P inhibits HDAC activity, resulting in increased acetylation of histones including histone H3 on Lys9 (H3K9ac; ref. 16). We confirmed these data, showing that SK2 inhibition reduced the amount of S1P associated with HDAC2 (Supplementary Fig. S10). c-Myc protein expression was concomitantly reduced following knockdown of SK2 by siRNA (Supplementary Fig. S11). Despite recent reports of increased Sphk1 expression in the presence of Sphk2 (31), SPHK1 gene expression was not increased by SK2 inhibition as determined by microarray analysis.

We questioned whether changes in c-Myc-regulated genes could segregate SK2 inhibitor–treated ALL cells from their respective controls. To this end, we extracted three previously published c-Myc gene signatures (22, 32, 33) from the original and the validation microarray data sets. Unsupervised hierarchical clustering separated the SK2 inhibitor–treated cells from control-treated cells (Fig. 3E and Supplementary Fig. S12). Gene Set Enrichment Analysis (GSEA) demonstrated a significant negative enrichment of both the Bild and Schuhmacher c-Myc signatures in SK2-inhibited cells. The specificity of this effect was apparent from the lack of any consistent association between NF-κB or PI3K/AKT pathway members or SP1 targets (Fig. 3F and Supplementary Table S4) and the absence of these signatures in SK1–treated cells (P = 0.17 and 0.08, respectively; Supplementary Fig. S10). This suggests that regulation of MYC plays a significant role in the response of ALL cells to SK2 but not SK1 inhibition. Interrogation of the GSE28497 data set using the Schuhmacher and colleagues’ (33) c-Myc signature found c-Myc targets to be increased in diagnostic patient samples (P = 0.019) consistent with the overexpression of MYC in these patient samples (Fig. 3G).
significantly reduced following inhibition of SK2 (Fig. 4F) consistent with their reduced expression.

**Concurrent Bcr/Abl and SK2 inhibition produces synergistic effects in Ph\(^+\) ALL**

New therapies for ALL will almost certainly be used in combination with established chemotherapy regimens. No significant interactive effects between SK2 inhibition and vincristine or doxorubicin were observed, with the fractional product method showing at best additive effects (Fig. 5A and Supplementary Fig. S14). In contrast, synergistic cell death was observed at all time points when inhibition of SK2 was combined with the proteasome inhibitor bortezomib (Fig. 5B).

Imatinib inhibited proliferation, but had little effect on the viability of Ph\(^+\) ALL cell lines (Fig. 5C and D). However, known resistance-inducing mutations in *BCR/ABL* were not detected. Resistance to imatinib-induced cell death was overcome by concurrent SK2 inhibition and clear synergistic interactions were observed in almost all conditions tested (Fig. 5E and Supplementary Fig. S14).

**SK2 inhibition is effective in a human xenograft model of B-lineage ALL**

Intraperitoneal injection of nonobese diabetic/severe combined immunodeficient (NOD/SCID) IL2\(\gamma_{c}^{-/-}\) mice with ABC294640 reduced plasma S1P concentrations by 23% after 2 hours, with a further decrease (45%) by 6 hours, and levels remained significantly suppressed for at least 24 hours (Supplementary Table S5). This indicates that ABC294640 significantly inhibits SK2 activity, reducing S1P plasma levels in vivo, consistent with the findings by Beljanski and colleagues (35). NOD/SCID IL2\(\gamma_{c}^{-/-}\) mice engrafted with human ALL cells were treated with 100 mg/kg/day ABC294640 or vehicle by intraperitoneal injection for 21 days, after which all animals were sacrificed. The percentage of ALL cells in the BM and blood was significantly reduced in SK2 inhibitor--treated mice, whereas the contribution to the spleen was...
significantly reduced in xenograft 1999 only (Fig. 6A–C, top). Average absolute levels of leukemia in the BM of SK2 inhibitor–treated mice were reduced by between 40% and 60% ($P < 0.004$; Fig. 6A, bottom). Reductions in the blood were between 60% and 67% ($P < 0.002$) and in the spleen by 38% and 79% ($P < 0.0001$) for xenografts 1999 and 1345 (Fig. 6B and C, bottom). Overall, inhibition of SK2 decreased the level of disease in all xenografts between 40% and 78% ($P < 0.00005$; Fig. 6E). No overt toxicity was noted, with the murine white blood cell (WBC) count being higher in SK2 inhibitor–treated animals bearing xenograft 1999 ($P = 0.018$), consistent with reduced disease, and not significantly different in the other xenografts (Fig. 6D).

Inhibition of SK2 significantly slowed the increase in circulating ALL cells, prolonging the survival of mice bearing xenograft 1999 ($P = 0.0012$; Fig. 6F and G) from a median of 30 days for control mice [95% confidence intervals (CI), 29–30 days] to 41 days (95% CI, 32–38 days) for the treated group. Concurrent inhibition of SK2 and Bcr/Abl with imatinib also significantly improved the survival of mice engrafted with a Ph$^+$ ALL (2070; Fig. 6G). In this experiment, the median survival of control mice was 49 days (95% CI, 47.5–50.5 days) with imatinib extending survival to a median of 65 days (95% CI, 63.4–96.6 days). Concurrent inhibition of SK2 further extended the survival to a median of 104.5 days (95% CI, 91.1–117.9 days; $P = 0.004$ compared with imatinib alone and $P = 0.044$ compared with ABC294640 alone).

**SK2 contributes to ALL development in mice**

Twenty-two of 29 mice receiving BCR/ABL–transduced B-cell progenitors from WT animals developed ALL, with a median survival of 42 days (95% CI, 32–52 days). Absence of SK2 reduced the incidence of BCR/ABL–driven ALL to 16 of 29 animals (median survival of 58 days; 95% CI, 47–69 days; $P = 0.002$; Fig. 7A). In contrast with reports by Liang and colleagues (31), the absence of SK2 did not result in increased SK1 expression (Supplementary Fig. S4B). The presence of BCR/ ABL and the expected deletion of Sphk2 were confirmed by PCR in all murine leukemias examined (Fig. 7B and C). All leukemias demonstrated a B-cell progenitor phenotype (Fig. 7D), with the majority being pre-B-ALL, lacking CD43 expression. Lymphoblasts were present in blood films and livers from all animals.

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**Figure 5.** (Continued.) The indicated cell lines were incubated with increasing concentrations of imatinib for 72 hours and assessed for proliferation (C) or survival (D). The mean and SD of four (C) and two (D) experimental replicates is shown. E, the indicated cell lines were cultured with vehicle or 50 μmol/L ABC294640 with or without the addition of 5 μmol/L imatinib for 48 hours or 72 hours and analyzed for viability. The mean and SD of two experimental replicates is shown. A, B, and E, hatched bar, calculated additive effect based on the effect of each agent alone; #, synergistic interaction between the agents as determined by a fractional product method.
Cells recovered from the spleens of leukemic mice demonstrated reduced c-Myc expression in leukemias arising in SK2**+/−** cells as compared with those derived from WT B-cell progenitors (Fig. 7G). These data demonstrate a role for SK2 in the development of **BCR/ABL**–driven ALL. ALL cells recovered from mice receiving **BCR/ABL**–transduced WT B-cell progenitors were sensitive to ABC294640, whereas those from SK2**+/−** B-cell progenitors were resistant, confirming that...
the cytotoxic effects of ABC294640 were mediated through SK2 (Fig. 7H).

Discussion

The oncogenic role of SK1 has been demonstrated in a range of malignancies; however, the role of SK2 in cancer is largely unknown. Overexpression of SK2 can be proapoptotic via binding to BclXL (36) and by its role in sphingolipid metabolism, resulting in increased levels of proapoptotic ceramide (37). Recently, sphingolipid metabolism was shown to be required for activation of BAK and BAX and apoptosis induction (38). However, recent studies have demonstrated a role for SK2 in tumor promotion (11, 35) and demonstrated that inhibition of SK2 can inhibit the in vitro and in vivo growth of the myeloid leukemia cell line, U937 (39). We examined the effects of Sphk2 deletion in the development of BCR/ABL–driven ALL, and SK2 inhibition in ALL cells in vitro and in vivo. Despite a recent report showing increased SK1 in Sphk2−/− mice (31), SK1 was not increased in Sphk2−/− ALL cells or following SK2 inhibition (Supplementary Fig. S4). We also explored the mechanism by which SK2 inhibition mediates its anticancer activity, revealing suppression of c-Myc as a key mechanism.

A role for SK2 in ALL biology was suggested by the presence of a gene signature consistent with increased SK2 activity in a large cohort of pediatric ALL samples collected at diagnosis. This was supported by the reduced incidence of ALL in a BCR/ABL–driven model of murine ALL in the absence of Sphk2, suggesting that SK2 plays a role in disease initiation in this setting. However, the efficacy of SK2 inhibition in established human leukemia indicates that it is also important in disease progression. Overall, this is the first report demonstrating the importance of SK2 in ALL.

Although overexpression of SK2 has been reported to produce antiproliferative effects and result in cell death, it is clear that SK2 can also promote cell growth. The reasons for these discrepant effects are not entirely clear. The subcellular localization of SK2 may be important for the ultimate function of this enzyme (40). A recent study showed that siRNA knockdown of SphK2 inhibited breast cancer cell proliferation despite increasing S1P production (41), speaking strongly to the importance of the localized activity of these enzymes over global S1P production. When associated with the endoplasmic reticulum, SK2 induced apoptosis via production of ceramide (42), whereas translocation to the cytoplasm resulted in survival-promoting effects in colon cancer cells (43). SK2 also has demonstrated protective effects at the mitochondria following ischemia reperfusion injury in a number of tissues including cardiomyocytes (44). In this study, it seems that the nuclear localization of SK2 and its known effects on histone H3-HDAC1/2 (16) were the dominant mechanism of action. In...
ALL cells, SK2 increases expression of oncogenic c-Myc, presumably through localized SIP production, promoting survival and proliferation. Consistent with this, ALL patient samples have increased MYC expression, increased expression of c-Myc–regulated genes, and are killed by c-Myc inhibition. Furthermore, exogenous expression of MYC resulted in a degree of resistance to SK2 inhibition by ABC294640.

SK2 inhibition has been associated with both caspase-dependent (45) and -independent cell death (46), with autophagy being involved in the latter. Autophagy also has cell survival roles (47) and although SK2 inhibition induced autophagy in ALL cells, this was not the cause of cell death, a situation similar to that following FTY720 exposure (48). Consistent with the lack of apoptosis, genes involved in apoptosis were not overrepresented in the microarray data (data not shown). Inhibition of SK2 has been shown to synergize with chemotherapeutic agents in breast cancer cell lines in vitro, via inhibition of NF-κB–mediated survival signals (49). However, our attempts at combining conventional chemotherapeutic agents such as vincristine and doxorubicin were disappointing, with no significant synergy seen. The lack of an effect on NF-κB signaling in ALL cells when SK2 is inhibited (Supplementary Table S4) may explain this result. We, therefore, turned our attention to the biologic response modifiers bortezomib, after reports of efficacy in lymphoid malignancies (50, 51), and found the combination of ABC294640 with this agent to be superior to conventional drugs. In addition, inhibition of SK2 also synergized with imatinib in Ph+ disease, reminiscent of work published with SK1 inhibitors (7). Overall, this suggests that combination treatments including SK2 inhibitors may have potential as therapeutic strategies.

A major novel finding in this study is the downregulation of the MYC gene and c-Myc target genes as a result of SK2 inhibition. This effect on MYC expression was specific for SK2 with SK1 inhibition failing to influence expression of MYC or c-myc target genes in ALL cells. Increased MYC and c-Myc target gene expression in ALL samples from patients and the ability of a c-Myc inhibitor to kill ALL cells provides evidence for a role for c-Myc in ALL cell biology. These data potentially explain the often opposing roles of SK2 in cell growth and survival, inhibiting growth and survival via effects on p21 and fos, while promoting these features by increasing expression of c-Myc. They also highlight a potentially underappreciated role of c-Myc in ALL biology and identify potential therapeutic strategies for the treatment of ALL and other c-Myc–driven malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.T. Wallington-Beddoe, S.M. Pitson

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