Metabolic Convergence on Lipogenesis in RAS, BCR-ABL, and MYC-driven Lymphoid Malignancies

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Metabolic Convergence on Lipogenesis in RAS, BCR-ABL, and MYC-driven Lymphoid Malignancies

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Abstract

Background

Metabolic re-programming is a central feature in many cancer subtypes and a hallmark of cancer. Many therapeutic strategies attempt to exploit this feature, although often having unintended side effects on normal metabolic programs and limited efficacy due to integrative nature of metabolic substrate sourcing. Although the initiating oncogenic lesion may vary, in lymphoid malignancies tumor cells often share similar environments and potentially similar metabolic profiles.

Methods

We searched publicly available data sets for common metabolic convergence in lymphoma and leukemia. Using *in vitro* cell lines derived in from conditional MYC, RAS, and BCR-ABL transgenic murine models we examine metabolic profiles of lipogenesis. We then utilize preclinical murine models and transgenic primary model of T-ALL to determine the effect of lipogenesis blockade across BCR-ABL, RAS, and c-MYC-driven

lymphoid malignancies. Statistical significance was calculated using unpaired T tests and one-way ANOVA.

Results

We find that de novo lipid biogenesis is a shared feature of several lymphoma subtypes. Using cell lines derived in from conditional MYC, RAS, and BCR-ABL transgenic murine models we demonstrate shared responses to inhibition of lipogenesis by the acetyl-coA carboxylase inhibitor 5-(Tetradecloxy)-2-furic Acid (TOFA). We identify specific cell death responses to TOFA *in vitro* and *in vivo* and demonstrate delayed engraftment and progression *in vivo* in transplanted lymphoma cell lines. We also observed delayed progression of T-ALL in a primary transgenic mouse model upon TOFA administration. In a panel of human cell lines, we demonstrate sensitivity to TOFA treatment as a feature of MYC^{high} expressing lymphoma cell lines.

Conclusions

These studies indicate that inhibition of lipogenesis may be a common survival strategy for many lymphomas and that high MYC expression is predictive of sensitivity to blockade of fatty acid synthesis.

Trial Registration

This study does not include any clinical interventions on human subjects

Keywords: c-MYC, BCR-ABL, RAS, lymphoma, T-ALL, cancer metabolism, lipogenesis, fatty acid synthesis, oncogene addiction

Introduction

Cancer cells can be defined by their constant and uncontrolled proliferation. This requires increased need for cellular building blocks such as amino acid synthesis,

nucleotide production, and *de novo* lipid synthesis (1, 2). To achieve this, cancer cells must reprogram their metabolic pathways to accumulate intermediates as sources of these building blocks. Thus, altered metabolism is a hallmark of cancer (3). For example, glucose metabolism is commonly altered to decouple glycolysis from pyruvate oxidation (Warburg effect) (4, 5). Although glycolysis is much less energy efficient than aerobic respiration, yielding 2 ATP instead 32 ATP, it provides a surplus of metabolic substrates that aerobic respiration does not, thereby providing a cellular growth advantage to cancer cells (6, 7). These substrates are utilized in additional metabolic pathways to provide the building blocks required for rapid proliferation. Additionally, lactate production from glycolysis has been shown to alter the intracellular redox balance thereby promoting invasiveness (5, 8, 9).

Efforts have been made to exploit these metabolic dependencies ever since the Warburg effect was first described (5, 10, 11). Despite the identification of several therapeutic targets, most glycolysis inhibitors show toxicity in normal tissues and/or limited therapeutic response (10). Lipid metabolism is altered in cancer cells where intermediate substrates from glycolysis are diverted from energy production to biosynthesis of fatty acids (5, 12, 13). These fatty acids are utilized to create membranes and signaling molecules such as phospholipids, sterols, and other lipids (5, 14, 15). All of these *de novo* lipid production pathways begin with the committed rate-limiting step involving conversion of acetyl-CoA to malonyl-CoA, facilitated by acetyl-CoA carboxylase (ACC or ACACA)(16, 17). In this study, we utilize cell lines derived from conditional murine models that drive several cancer subtypes (18, 19). This allows for the conditional expression of RAS, MYC, and BCR-ABL controlled by a Tet-O system where the addition of doxycycline halts expression, permitting perturbation studies of the driving oncogene (20). We utilized cell lines derived from these models to explore common metabolic

features in the context of MYC, RAS, and BCR-ABL-driven lymphomas. Despite being driven by different oncogenes, we found increased lipid biogenesis in all three models. Cancer cells have previously been shown to be sensitive to lipogenesis inhibitors, although oncogene-driver susceptibility has not been examined closely (21). In this study, we examine the effects of ACACA inhibition in lymphomas using 5-(Tetradecloxy)-2-furic Acid (TOFA) resulting in shutdown of lipid biogenesis (17, 22). We find that although each driver oncogene is sensitive to blockade of lipogenesis and MYC-addicted cells are particularly responsive, providing a rationale for examining high MYC expressing lymphomas for sensitivity to inhibitors of the fatty acid synthesis pathway.

Results

MYC, BCR-ABL, and RAS regulate lipogenesis

We began by examining lipogenesis gene regulation in several blood cancers using publicly available data sets. In an *Eμ-Myc* model (23) that drives B-cell lymphoma, we observed that ACACA and FASN were upregulated during lymphoma progression driven by MYC (Fig. 1A). Both ACACA and FASN are committed steps in facilitating metabolic fate early in the fatty acid synthesis pathway. Recent studies in other cancers have shown reliance on the lipogenesis pathway (24-29). To determine if the lipogenesis dependency is a general feature of lymphoma, we examined other oncogene driven models such as BCR-ABL and RAS. Inhibition of BCR-ABL with Imatinib in human BCR-ABL-driven cell lines (30) show reduction in lipogenesis gene expression (Fig. 1B) (GSE 23743), and with the exception of one cell line they also display reduced MYC expression when treated with Imatinib. The loss of MYC is also correlated with reduction in key lipogenesis genes. The MYC^{high} BCR-ABL line BV173 showed increased expression of those same genes (Fig. 1B), suggesting that even in BCR-ABL driven lymphoma cell

lines, MYC expression may be linked to the expression of key lipogenesis genes. Using cell lines derived from the *Eμ-tTA/Tet-O-MYC* conditional transgenic murine model (31), we explored genome-wide transcriptional changes when MYC expression is revoked over several time points. Examination of RNA-seq data for key lipogenesis genes shows a general reduction of expression patterns when MYC expression is abrogated (Fig. 1C). Additionally, recently published data (32) of the primary model compared to background strain and MYC on/off conditions show upregulated lipogenesis genes *in vivo* (Supplemental Fig. 1A).

Examination of ChIP-seq data in the human Burkitt lymphoma line P493-6 (33), which also features conditional MYC expression, reveals direct MYC binding at promoter regions of lipogenesis genes (Supplemental Fig. 2A). Using ChIP-seq data of U2OS cells expressing conditional MYC (26) also observed direct binding of Myc to ACC, but not to other lipogenesis genes, suggesting that Myc occupancy is coordinated by tissue subtype (Supplemental Fig. 2B), not a general feature of c-Myc biology.

We obtained several cell lines derived from transgenic mouse models of lymphoma that are driven either by MYC, RAS or BCR-ABL. The expression of the oncogene is controlled by the Tet-O system where the addition of doxycycline suspends gene expression. Using these cell lines we examined expression of fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD), genes that are involved in the early and late stages of the fatty acid synthesis pathway respectively. Examination of the MYC on versus off state reveals MYC positively regulates expression of this pathway (Fig. 1D). Interestingly, we see common mechanism of metabolic regulation in the RAS and BCR-ABL driven cell lines, suggesting a potential common metabolic pathway in lymphoma (Fig. 1D). Taken together, these results suggest that MYC-driven lymphoma cells upregulate lipid biogenesis pathways, which may be a common feature across lymphoid malignancies.

Inhibition of Lipogenesis by TOFA suppresses cell proliferation.

To determine whether MYC-driven cancers are sensitive to blockade of the lipogenesis pathway we utilized 5-(Tetradecloxy)-2-furic Acid (TOFA), an inhibitor of acetyl-CoA carboxylase 1 (ACACA)(17, 22). TOFA blocks the synthesis of malonyl-CoA and thereby inhibits fatty acid synthesis pathway. We treated several MYC-addicted cell lines derived from conditional transgenic models of T-ALL with TOFA, which reduced viable cell populations (Fig. 1E). We also examined the P493-6 conditional MYC expressing cell lines that had revealed MYC dependent binding at promoter regions of lipid biogenesis genes (Supplemental Fig. 2A). The P493-6 lymphoma cells also show dependency upon lipid biogenesis with TOFA resulting in reduced cell numbers (Fig. 1E). We also examined the effects of TOFA in RAS and BCR-ABL driven tumor cell lines, also derived from transgenic animal models. There was a similar dose dependent reduction in cell populations in these lines (Fig. 1E), again suggesting general convergence on metabolic pathways in lymphomas and leukemia.

Inhibition of Lipid biogenesis and ER stress

Several studies have linked changes in lipid metabolism to endoplasmic reticulum stress (ER stress) and unfolded protein response stress (UPR stress) (34-39). We examined the genes that are upregulated in response to UPR stress in TOFA treated cells (Supplemental Fig. 3A). The C/EBP homologous protein (CHOP) transcript is activated by endoplasmic reticulum stress (ER stress) and was upregulated in each of the MYC, RAS, and BCR-ABL driven cell lines, although the strongest response is seen in the MYC-dependent cell lines. MYC-dependent cells also display upregulation of activating transcription factor ATF6 α , a transcription factor that activates UPR stress

target genes (36). The BCR-ABL-driven cell lines show additional upregulation of the key chaperone HSP- α 5, involved in degradation of misfolded proteins, and XBP1, which is activated by accumulation of unfolded proteins in the ER. The RAS-driven lymphoma cell lines actually show a downregulation of XBP1, ATF6 α , and HSP- α 5. These data indicate that while UPR stress can be associated with lipogenesis in MYC and BCR-ABL-driven cell lines, RAS cell lines actually downregulate the UPR stress response and therefore UPR stress is not a common feature of oncogenic lipogenesis in lymphoid malignancies.

We next injected luciferase-labeled MYC cell lines into recipient NOD-SCID IL-2R $\gamma^{-/-}$ (NSG) mice and treated the mice with either TOFA or DMSO. When the mice were moribund due to disease burden, we collected spleens from euthanized animals and examined UPR stress genes *in vivo* (supplemental figure 3B). We observed slight upregulation of UPR stress genes in TOFA-treated mice compared to control. Interestingly, we also observed a reduction of the transgenic MYC, suggesting increased clearance of MYC-addicted cells in the presence of TOFA, and potential explanation for mitigated UPR stress response *in vivo* with exogenous cell origins, especially considering the robust increase in lipogenesis genes seen in the primary model (Supplemental Fig. 1A).

Inhibition of Lipogenesis leads to apoptosis

To further examine the reductions in cell populations upon TOFA treatment (Fig. 1E), we measured cell death using flow cytometry. Co-stains for 7-AAD and Annexin V reveal an increase in cell death in MYC-driven T-ALL (Fig. 2A). Again, we observed similar effects in RAS and BCR-ABL driven lymphomas, although the most potent effects remain in the MYC-driven T-ALL (Fig. 2A).

Our *in vivo* results suggesting clearance of MYC-addicted cells (Supplemental Fig. 3B) upon TOFA treatment along with increased cell death observed *in vitro* (Fig. 2A) suggest apoptosis programs may be active *in vivo*. We next characterized apoptosis in spleens taken from mice that had been implanted with a MYC-addicted T-ALL tumor cell line and treated with TOFA for 4 days. In these samples, we detected a potent increase in the apoptosis marker cleaved caspase-3 in the TOFA treated animals (Fig. 2B), confirming *in vivo* sensitivity of MYC-addicted cells to lipogenesis blockade.

We next asked whether proliferating lymphocytes were broadly susceptible to TOFA treatment, or whether this phenomenon was specific to tumor cells. We next harvested spleens from the wild-type background FVB/N mice and activated splenocytes with concanavalin A (ConA), and treated cells with TOFA. For activated splenocytes, TOFA does not cause a significant reduced proliferation or cell density (Fig. 2C), whereas cisplatin, used as a positive control for its effects on ConA-treated splenocytes, did (Fig. 2C). Taken together, these data indicate that MYC-dependent cells are sensitive to lipogenesis pathways for survival both *in vitro* and *in vivo*, and general cell death in lymphocytes is not a due to TOFA treatment.

Inhibition of lipogenesis delays engraftment, progression and splenic infiltration

We next considered the possibility that inhibition of lipogenesis may delay engraftment and cancer progression *in vivo*. We implanted MYC-driven luc-labeled tumor cells derived from the primary transgenic mouse model in recipient mice. TOFA treated mice exhibited reduced tumor burden as measured by bioluminescent imaging as compared with the DMSO treated mice (Fig. 4A), confirming that MYC-addicted cells are metabolically sensitive to lipogenesis inhibition. Spleen size at terminal endpoint was measured to determine effects of TOFA treatment on infiltration of T-ALL cells (Fig. 5C).

TOFA treated mice exhibited a reduced spleen size compared to DMSO treated mice, indicating reduced T-ALL disease burden in this disseminated mouse model.

We then subcutaneously injected BCR-ABL and RAS dependent cell lines and the MYC-dependent Burkitt lymphoma cell line P493-6 into NSG mice. In all three oncogene dependent cell lines, TOFA treatment reduced tumor progression (Fig. 5B). In the P493-6 MYC-dependent cell lines the disparity between control and TOFA treated mice was the most striking, such that half the cohort of control treated mice required sacrificing before experimental endpoints had been achieved for TOFA treated mice. These results suggest a common convergence on lipogenesis for lymphoma cells, and the MYC-dependent cells are particularly sensitive to disruption of this pathway.

Using the *Eμ-tTA/Tet-O-MYC* primary transgenic mouse model, mice were treated at five weeks of age just prior to visible onset of disease with either TOFA or DMSO for one week. The mice were then tracked for an additional 4 weeks with no treatments and sacrificed when control mice developed T-ALL as determined by hunched posture, enlarged lymph nodes, and ruffled fur. Survival data at 4 weeks post injection with TOFA was sufficient to show delayed progression of the primary transgenic model of MYC-dependent T-ALL.

MYC expression determines response to TOFA in human cell lines

We next examined the effects of lipogenesis inhibition on human cell lines. Previously we showed that the MYC-conditional Burkitt's lymphoma cell line P493-6 was sensitive to TOFA treatment (Fig. 1E, 3B). To expand upon these observations, we obtained a panel of eight human T-ALL and Burkitt lymphoma cell lines and examined relative MYC levels (Fig. 4A). We grouped the cell lines into five MYC^{high} expressing cell lines and three MYC^{low} cell lines. Cell viability was greatly reduced in TOFA treated cells

that had high MYC expression, but not in cell lines that had reduced levels of expression (Fig. 4B, supplemental Fig. 4A). The lack of sensitivity in the low MYC expressing cells indicate that TOFA treatment is not a general disruptor of the cell cycle through its metabolic influence in all lymphomas and that MYC-driven lymphomas are particularly sensitive to blockade of lipogenesis (Fig. 4C).

Discussion

We examined the convergence upon lipid biogenesis in RAS, BCR-ABL and MYC driven lymphoma cells in culture. All three oncogene driven models are sensitive to lipid biogenesis inhibition, however MYC-driven cell lines appear to be the most reliant upon this pathway. Indeed, in human cell lines sensitivity to TOFA is tightly linked to MYC expression. When these cell lines were injected into NSG mice, the resulting tumors were sensitive to lipogenesis inhibition. Using published data we also observed direct MYC binding to lipogenesis genes in the Burkett's lymphoma model cell line P493-6, but not in conditionally expressing MYC osteosarcoma cell lines, suggesting that sensitivity to lipogenesis is not a generalizable MYC hallmark, but rather may be a general program employed by lymphoid malignancies. In the oncogene dependent lymphoid cancer cell lines, MYC-driven models were particularly sensitive to lipogenesis inhibition. We examined the relationship between the UPR stress response and find that this response is not necessary for sensitivity to lipogenesis inhibition. The lack of the transgene detected compared to vehicle treated mice suggests that the MYC cells injected into the mice were transcriptionally absent in the TOFA treated tumor cells in the spleen, and the significant increase in cell death suggests that the MYC-dependent population was actively undergoing apoptosis *in vivo*.

In contrast, the primary model (*Eμ-tTA/Tet-O-MYC*) treated with TOFA significantly delayed disease progression (Fig. 3D). Previously published experiments using this model in a MYC on vs MYC off vs murine background clearly shows a preference for lipogenesis pathway upregulation in a MYC positive state (32). Thus, in high MYC expressing lymphomas, reliance upon fatty acid synthesis could be considered a metabolic liability.

Although murine models are metabolically dissimilar to human metabolism, human cell lines recapitulate the sensitivity to blockade of ACACA function (Fig. 6A and 6B) dependent upon MYC expression levels. These results together suggest that cells with high MYC expression have exploited a metabolic strategy that relies upon fatty acid synthesis. Therefore, this metabolic feature is specifically driven by MYC and may provide rational for development of more specific inhibitors and treatment strategies.

Conclusion

These studies indicate that inhibition of lipogenesis may be a common survival strategy for many lymphomas and that high MYC expression is predictive of sensitivity to blockade of fatty acid synthesis, and a potential therapeutic strategy.

List of Abbreviations: MYC: c-MYC, ER stress: endoplasmic reticulum stress, UPR stress: unfolded protein response stress, ACACA (or ACC): acetyl-CoA carboxylase, NSG: NOD-SCID IL-2Rg^{-/-} mice, TOFA: 5-(Tetradecloxy)-2-furic Acid, MYC on: no doxycycline, MYC off: 20ng/ml doxycycline

Declarations

Ethics approval and Consent to Participate

Studies did not involve human subjects. Special consideration was given to minimization of pain and distress for the humane and ethical treatment of the animals utilized for the study. In addition to coordinating with APLAC protocols and veterinarians and obtaining IACUC approval, each experiment was considered for whether the number of mice utilized is appropriate, and consideration of humane endpoints to reduce pain and distress.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analyzed during the current study are available on the central repository GEO: GSE 51011, GSE 23743, GSE 36354, GSE 44672. The RNA-seq timecourse utilized in Fig. 1C is currently not available publicly, but will be deposited in GEO upon publication and are available from the corresponding author upon written request.

Competing interests

The authors declare that they have no competing interests

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Authors contributions

DL: Conceptual approaches. Data mining experiments, RNA-seq experiments. Experimental design, implementation and analysis used to generate all figures. Preparation of Manuscript. SS: Conceptual approaches, and project guidance. Data mining experiments. Generation of luciferase labeled cells utilized for in vivo BLI experiments. Project guidance and feedback SCC: Experimental design and implementation of in vitro experiments. Project guidance and feedback MR, and IL: Independent validation of the seminal findings, project guidance and feedback. AM: performed in vivo experiments. Project guidance and feedback. GD: performed in vitro experiments and technical and analytical support to DL

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METHODS

Cell culture conditions

Cells were cultured in RPMI with L-glutamine (Gibco) supplemented with 10% fetal bovine serum (Tissue Culture Biologicals) with penicillin/streptomycin (Gibco) in a humidified 5% CO₂ atmosphere. For mouse cell lines 50uM beta-mercaptoethanol was added to the media. Cells were maintained at 37 °C in a humidified incubator with 5% CO₂, and typically passaged every three days.

RNA extraction & cDNA synthesis

RNA extraction from 2×10^7 cells is done using the Qiagen RNEasy Extraction kit. RNA quality and concentration are assessed by a spectrophotometer, the Nanodrop. cDNA is then synthesized from 0.4 µg of the extracted RNA using Qiagen cDNA reverse transcription kit. The cDNA is then stored at -20°C.

qPCR

Primers are designed by using NCBI PrimerBlast program and primer specificity was then verified using BLAST. Primers were generated by the Stanford PAN facility. Real-time PCR is performed in 96-well plates on an ABI Biosystems Thermo Cycler 7500. All primers are detected by using SYBR Green as fluorophore. Reactions are carried out in 20 µl that contained 1.5 µl cDNA, 0.5 µM forward and reverse primers and 8uL water and 10 µl of 2× SYBR Green master mi (ABI). Amplification cycle is as follows: 95°C for 3 min, 35 cycles of 95°C for 10 s, 63°C for 30 s, 72°C for 30 s and a final extension at 72°C for 5 min. At the end of the amplification cycles, a dissociation curve is done to verify non-specific amplification. The thermal cycler software generated threshold cycle (Ct) values for each gene; Ct is the number of cycles required to reach the threshold fluorescence 15 standard deviations above the noise. The Ct values are exported into Excel for analysis, and GraphPad Prism for statistical analysis

Primer Table:

Gene

hMYC	Forward	CTGCGACGAGGAGGAGAACT
hMYC	Reverse	GGCAGCAGCTCGAATTTCTT
Chop	Forward	CTGGAAGCCTGGTATGAGGAT
Chop	Reverse	CAGGGTCAAGAGTAGTGAAGGT
Xbp1 spliced	Forward	GACAGAGAGTCAAACCTAACGTGG
Xbp1 spliced	Reverse	GTCCAGCAGGCAAGAAGGT
Atf6-alpha	Forward	AGCGCCCAAGACTCAAACC
Atf6-alpha	Reverse	CTGTATGCTGATAATCGACTGCT
Atf4	Forward	ATGGCGCTCTTCACGAAATC
Atf4	Reverse	ACTGGTCGAAGGGGTCATCAA
Eif2a	Forward	TACAAGAGACCTGGATACGGTG
Eif2a	Reverse	TGGGGTCAAACGCCTATTGATA
Scd1	Forward	TTCTTGCGATACTCTGGTGC
Scd1	Reverse	CGGGATTGAATGTTCTTGTCGT
Fasn	Forward	GGAGGTGGTGATAGCCGGTAT
Fasn	Reverse	TGGGTAATCCATAGAGCCCAG
Ubc	Forward	AGCCCAGTGTTACCACCAAG
Ubc	Reverse	ACCCAAGAACAAGCACAAGG

RNA-seq

MYC-dependent cells derived from the primary *Eμ-tTA/Tet-O-MYC* murine model and maintained in culture. 100 X 10⁶ cells were seeded in T175 flasks in fresh media and timepoint samples began 24hr post seeding. Cells were dosed with 20ng/ml doxycycline and samples collected at the indicated time-points. RNA extraction included QIA shredder step, RNEasy kit, and on column DNA digestion (all reagents from Qiagen). Samples were shipped on dry ice to Beijing Genomics Institute (BGI) for the RNA-seq QC and sequencing pipeline and read analysis.

Cell Titer Glo Assay

Cells were seeded at 10,000 cells per well in 96 well format. 24 and 48 hours post seeding cells were treated as indicated. At 72 hours post seeding cells were assayed using CellTiter-glo assay (Promega G7570). After a 5 min incubation cell populations were measured using spectramax (Molecular Devices). Background values were subtracted

(media + CellTiter-glo) and average of 4 wells were normalized to Vehicle treated cells. Statistical significance was determined using the Student's T-test.

Annexin V and 7-AAD experiments

Cells were seeded in T25 flasks and treated as indicated. At 24hrs post seeding/post initial treatment, remaining cells were treated to obtain 24hr timepoint. At 48hrs post seeding/post initial treatment cells were harvested for analysis. Briefly, 1 million cell aliquots from each treatment were collected and washed twice in cold PBS, followed by centrifugation at 1300 RPM. Cells were resuspended in 1X Annexin V Binding Buffer (BD Pharmingen). Cells were then stained with either no stain, 7-AAD (BD cat# 51-68981E), or PE Annexin V (BD cat# 5165875X) or 7-AAD and Annexin V for one hour. Cells were then detected by flow cytometry (using BD FACS Aria SORP instrumentation). Data Analysis was performed using FlowJo (TreeStar).

Cleaved Caspase 3 immunostaining:

O.C.T. embedded tissue slides were prepared and stored at -20. Slides were thawed at room temp (RT) for 30 min, and incubated at -20 in acetone for ten minutes, followed by air drying (10 min). 1X PBS rinse, and Avidin block (Vector labs, Burlingame, Blocking kit cat# sp-2001) 10 min. Three minutes 3X PBS wash, 10 minutes Biotin block at RT. 3X PBS wash followed by serum free protein block (Dako cat # 2013-09) for 30 minutes at 37C. Cleaved Caspase 3 antibodies (cell signaling #9661S) 1:100 in Dako antibody diluent (Cat# S202230-2CN) overnight at 4C. 3X PBS washes were followed by biotinylated anti-rabbit (Vector Labs Cat# BA-1000) 1:300D for 30 min at RT, 3X PBS washes and 1:300 cy3-streptavidin (Vector Labs cat# BMK-2202) for 30 minutes at RT, 3X PBS washes and final wash with Vectashield (Vector Labs, Cat # H-1200) with DAPI followed by storage at 4C.

In Vivo Mouse Experiments

Cell lines generated from *Em-tTA/Tet-O-MYC* transgenic murine model of MYC addiction in T-ALL were transduced with PMSCV-luc-puro. Cells were counted and 2 million cells injected into NSG mice intravenously. 24 hours after injection, mice were treated as indicated and imaged each day for four days to detect total flux by bioluminescence imaging. For bioluminescence imaging, animals were anesthetized with inhaled isoflurane and oxygen using the Xenogen XGI-8 5-port Gas Anesthesia System. d-luciferin was injected intraperitoneally (150 mg/kg) 10 minutes prior to imaging. Animals were placed into the light-tight chamber and were imaged with an IVIS-200 cooled CCD camera (Xenogen). Living Image (Xenogen) was used to collect and analyze data and generate pseudocolor images. Treatment groups were evaluated for statistical significance using the student's T-test. The murine RAS, BCR-ABL cell lines were generated previously from conditional murine transgenic models.

Subcutaneous experiments: NSG mice were also subcutaneously injected with BCR-ABL, RAS-dependent cell lines derived from conditional murine models. In addition, the MYC-dependent Burkitt's lymphoma cell line P493-6 was also subcutaneously injected into NSG mice with 10,000 cells in PBS and treated as indicated. Tumors were measured every two days until tumor burden reached terminal endpoint, whereupon relevant tissue was collected.

Oncogene inactivation

Conditional MYC-driven mouse T-ALL cell lines were derived from *Em-tTA/tet-O-MYC* mice¹. c-MYC was inhibited in *Em-tTA/tet-O-MYC* T-ALL and P493-6 cells by treating cell cultures with 0.02 µg/ml doxycycline (Sigma-Aldrich, T7660) for indicated time-points. The RAS and BCR-ABL cell lines were also generated from conditional murine transgenic models maintained by the Felsher laboratory (Stanford, School of Medicine). The conditional cell lines were confirmed to be negative for mycoplasma contamination and

maintained in Roswell Park Memorial Institute 1640 medium (RPMI, Invitrogen) with GlutaMAX containing 10% fetal bovine serum, 100 IU/ml penicillin, 100 µg/ml streptomycin, 50 mM 2-mercaptoethanol at 37 °C in a humidified incubator with 5% CO₂.

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Figure Legends

Fig. 1. MYC regulates Lipogenesis Genes.

A) E μ -MYC model of B-cell lymphoma RNA-seq reveal upregulation of key lipogenesis genes linked to MYC expression as the B-cell lymphoma progresses. (GSE 51011) B) Microarray analysis of BCR-ABL dependent lymphoma cell lines show that inhibition of BCR-ABL results in downregulation of key lipogenesis genes (GSE 23743). C) RNA-seq was performed on cell lines derived from E μ -tTA/Tet-O-MYC transgenic model in a MYC off time-course. Decreased expression values were observed for fatty acid synthesis pathway genes (*data to be deposited upon publication*) upon MYC suppression. D) Cells derived from conditional transgenic murine models with differing oncogenic drivers. Conditional expression of the initiating oncogene is controlled by a Tet-Off system. The enhanced expression of MYC or BCR-ABL or RAS results in activation of the lipogenesis pathway as shown by QPCR for each cell type, indicating a common feature of T-ALL regardless of the initiating oncogene. Values were normalized to ubiquitin controls and

reported as fold change relative to MYC on condition (vehicle). * $P \leq 0.1$; ** $P \leq 0.01$; ** $P \leq 0.001$; *** $P \leq 0.001$; **** $P \leq 0.0001$ E) Cell populations in oncogene dependent lymphoid malignancies are sensitive to lipogenesis inhibition with TOFA, with MYC cells displaying the greatest cell population decline. Values were normalized to background controls and reported as percent cell population relative to vehicle control. * $P \leq 0.1$; ** $P \leq 0.01$; ** $P \leq 0.001$; *** $P \leq 0.001$; **** $P \leq 0.0001$

Fig. 2. Inhibition of Lipogenesis in T-ALL results in Cell Death.

A) TOFA treatment in either MYC, RAS, or BCR-ABL-dependent lymphoid results in significant increased cell death as detected by Annexin V and 7-AAD co-staining (quadrant 2) over time (5.5 $\mu\text{g/ml}$). B) NOD-SCIDIL-2Rg^{-/-} (NSG) mice were intravenously injected with MYC-driven T-ALL cells derived from *E μ -tTA/Tet-O-MYC* transgenic model, and treated with TOFA for 4 days, followed by 4 days off treatment. Cleaved caspase 3 is significantly more prevalent in splenic tissue from TOFA treated mice when compared to control. C) Spleens were harvested from the wild-type FVB/N mice (transgenic background), RBCs were lysed, and splenocytes activated with ConA, followed by indicated treatments. TOFA treatment did not significantly impair the activated splenocyte population compare to the ConA alone control. Comparison to ConA control utilized an unpaired T test ** $P \leq 0.01$

Fig. 3. TOFA treatment delays engraftment and splenic infiltration.

A) Luciferase labeled MYC-driven lymphoma cells from *E μ -tTA/Tet-O-MYC* conditional transgenic murine model were intravenously injected into NOD-SCIDIL-2Rg^{-/-} (NSG) and tracked using bioluminescent imaging (BLI). Mice treated with TOFA show significantly

decreased signal indicating reduced engraftment. B) RAS and BCR-ABL driven T-ALL cell lines were allografted subcutaneously into NSG mice. We observed significant reductions in tumor volume for mice treated with TOFA. We also observed significant reduction in tumor volume using the human B-cell lymphoma cell lines P493-6 subcutaneously allografted on NSG mice. C) Further analysis of the spleens from the mice utilized in panel A, we observed a significant reduction in spleen size in TOFA treated animals compared to vehicle control, suggesting decreased infiltration. D) *Eμ-tTA/Tet-O-MYC* primary model of T-ALL, mice were treated with TOFA at 5 weeks of age for one week and tracked for an additional 4 weeks. TOFA treated mice were less moribund and exhibited significantly reduced spleen sizes, suggesting inhibition of lipogenesis results reduced infiltration of the spleen. Comparisons to vehicle control utilized unpaired T test * $P \leq 0.1$; ** $P \leq 0.01$

Fig. 4: MYC expression determines response to TOFA in human lymphoma cell lines

Metabolic activity in mouse models and cell lines differ greatly from human cells. A) We obtained a panel of human lymphoma cells and measured relative MYC expression levels. The cell lines were grouped as either c-MYC high or c-MYC low. B) Metabolic cell activity was measured after 48hrs of TOFA treatment in the panel of human cell lines and ED 50 values were calculated. C) The sensitivity of the cell lines was tightly linked to MYC expression levels with all c-MYC high cells showing reduced populations, and c-MYC low cells unaffected by inhibition of Lipogenesis. Comparisons high vs low MYC expression utilized unpaired T test *** $P \leq 0.001$

Figure 1: Regulation of Lipogenesis in Lymphoma

Figure 2: Inhibition of Lipogenesis leads to cell death

Figure 3: TOFA Inhibits Growth *in vivo*

Figure 4: MYC expression determines response to TOFA in human lymphoma cell lines

Supplementary Figure legends

Supplementary Fig. 1. RNA-seq profiles of lipogenesis genes from *Eμ-tTA/Tet-O-MYC* model T-ALL.

A) Data mining experiments of MYC on vs off state in the *Eμ-tTA/Tet-O-MYC* primary model of T-ALL, inclusive of the FVB/N background shows MYC-dependent regulation of lipogenesis genes *in vivo*. GSE106078. Statistical analysis by ordinary 1-way ANOVA. *P≤0.1; **P≤0.01; ***P≤0.001; ****P≤0.0001

Supplementary Fig. 2. Direct MYC binding at lipogenesis genes.

A) ChIP-seq data shows direct MYC binding at promoters of the lipogenesis pathway upon increased MYC expression in Burkitt's like P493-6 cells (GSE 36354). B) ChIP-seq data in a conditional MYC expressing osteosarcoma cell line indicates that MYC-dependent regulation of lipogenesis is tissue dependent. (GSE 44672)

Supplementary Fig. 3. Unfolded protein response stress.

UPR stress response genes were monitored for changes in expression levels in response to lipogenesis blockade in MY, BCR-ABL, and RAS-dependent cell lines. Cells were treated with TOFA for 24hrs and expression levels of the indicated lipogenesis genes were monitored. Although UPR stress responses were modulated in each cell line, the expression levels do not appear to be related to the observed increase in lipogenesis. B) Cell lines derived from *Eμ-tTA/Tet-O-MYC* model were injected intravenously into NOD-SCIDIL-2Rg^{-/-} and tracked for engraftment. Splenic tissue was collected from moribund treated with either vehicle or TOFA as indicated, and mRNA levels were monitored. Comparisons to vehicle control utilized unpaired T test **P≤0.01; ***P≤0.001; ****P≤0.0001

Supplementary Fig. 4. Response to TOFA in human cell lines.

A) A panel of human lymphoid cell lines were treated with increasing doses of TOFA and cell populations were monitored (proliferation) via metabolic activity (cell titer glo assay, Promega)

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Figures

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