Phosphatidylinositol 3-Kinase-dependent Modulation of Carnitine Palmitoyltransferase 1A Expression Regulates Lipid Metabolism during Hematopoietic Cell Growth*3

Ralph J. DeBerardinis1,2,1, Julian J. Lum1,2, and Craig B. Thompson1,3

From the 1Abramson Family Cancer Research Institute, Department of Cancer Biology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104 and the 2Division of Child Development, Rehabilitation Medicine and Metabolic Disease, Department of Pediatrics, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania 19104

An abundant supply of extracellular nutrients is believed to be sufficient to suppress catabolism of cellular macromolecules. Here we show that, despite abundant extracellular nutrients, interleukin-3-deprived hematopoietic cells begin to catabolize intracellular lipids. Constitutive Akt activation blunts the increased β-oxidation that accompanies growth factor withdrawal, and in growth factor-replete cells, phosphatidylinositol 3-kinase (PI3K) signaling is required to suppress lipid catabolism. Surprisingly, PI3K and Akt exert these effects by suppressing expression of the β-oxidation enzyme carnitine palmitoyltransferase 1A (CPT1A). Cells expressing a short hairpin RNA against CPT1A fail to induce β-oxidation in response to growth factor withdrawal and are unable to survive glucose deprivation. When CPT1A is constitutively expressed, growth factor stimulation fails to repress β-oxidation. As a result, both net lipid synthesis and cell proliferation are diminished. Together, these results demonstrate that modulation of CPT1A expression by PI3K-dependent signaling is the major mechanism by which cells suppress β-oxidation during anabolic growth.

Single-cell eukaryotes like yeast autonomously regulate their uptake of nutrients from the extracellular environment. In such organisms, cellular metabolism is regulated primarily in response to nutrient availability (1). In contrast, it has been proposed that mammalian cells do not take up and metabolize nutrients without instruction from extracellular signals. These signals, which include cytokines and other lineage-specific growth factors, stimulate signal transduction pathways that orchestrate cellular metabolism through effects on gene expression and enzyme kinetics. Together, these signal-induced changes function to direct uptake and utilization of nutrients, channeling metabolites into biosynthetic pathways (2–4). One important example of this phenomenon occurs during lymphocyte stimulation, where receptor-induced signaling directly increases glucose transport, glycolysis, lactate production, and synthesis of lipids (5–9).

Many of the metabolic changes elicited by growth factors result from activation of the phosphatidylinositol 3-kinase (PI3K)/Akt signaling system. In this pathway, binding of a growth factor to its surface receptor induces activation of the lipid kinase PI3K, which phosphorylates phosphatidylinositol species (10). End products of PI3K activity recruit the serine/threonine kinase Akt to the plasma membrane where it becomes a substrate for phosphorylation and activation by phosphoinositide-dependent protein kinase 1 and other regulatory kinases (10). The metabolic effects of growth factor-induced PI3K/Akt stimulation include increases in glucose import and glycolysis, which fuel the bioenergetic and biosynthetic activities of growing cells (11–13). Constitutive activation of PI3K and/or Akt through a variety of genetic mechanisms is a common transforming event in human cancer, including some 25% of breast carcinoma and 30% of colon carcinoma (14–17). Cancer cells with constitutive Akt activity have a growth factor-independent ability to take up glucose and channel it into biosynthetic pathways, adopting a glucose-driven metabolism long known to characterize rapidly proliferative tumors (18, 19). Therefore, Akt activation supports anabolic metabolism in non-transformed cells and contributes to autonomous growth in tumor cells.

The net synthesis of lipids, particularly phospholipids for daughter cell membranes, is required for cell proliferation. Inhibition of lipid synthesis is an effective strategy to suppress proliferation of various cell types (20, 21). Recent evidence has shown that stimulation of PI3K/Akt increases cellular synthesis of fatty acids from glucose and other precursors (22–24). Fatty acids are important metabolic intermediates, because they can either be used for lipid synthesis and protein modification (e.g. palmitoylation, myristoylation, and synthesis of glycerophosphatidylinositol anchors), or they can be degraded through mitochondrial...
β-oxidation, which produces substrates that maintain ATP generation through oxidative phosphorylation. In the liver, the rate of β-oxidation is determined by the malonyl-CoA-dependent allosteric regulation of carnitine palmitoyltransferase I (CPT I), an enzyme located on the outer mitochondrial membrane that esterifies long chain fatty acids to carnitine, thereby initiating mitochondrial import (25–28). Such dynamic regulation of lipid synthesis/degradation allows hepatocytes to oscillate between postprandial lipid synthesis and fasting-induced lipid degradation.

Whether lipid metabolism is similarly regulated in cells undergoing a growth factor-induced proliferative response has not been investigated. Therefore, we have examined the regulation of β-oxidation during cell growth and survival of hematopoietic cells. In the quiescent state, hematopoietic cells oxidize fatty acids obtained from the extracellular milieu and from intracellular lipids, and these activities allow them to survive glucose withdrawal. When stimulated to proliferate, hematopoietic cells commit to net lipid synthesis by suppressing β-oxidation and concomitantly inducing lipid synthesis. The ability to suppress β-oxidation is required for these cells to achieve maximal rates of proliferation. Surprisingly, this suppression results primarily from modulation of CPT1A expression rather than its enzyme activity. These results identify a novel mechanism used to modulate lipid metabolism in proliferating cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Reagents**—IL-3 dependent, bak−/− bak−/− cells have been described (29) and were used here to avoid the confounding variable of apoptotic cell death. In other experiments, IL-3-dependent FL5.12 cells were studied. The IL-3-dependent, bak−/− bak−/− cells were cultured in RPMI 1640 medium containing l-glutamine (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Gemini); 3 ng/ml recombinant murine IL-3 (BD Pharmingen); HEPES buffer (9 mM, Invitrogen); β-mercaptoethanol (50 μM, Sigma); penicillin G and streptomycin sulfate (91 units/ml and 91 μg/ml, respectively, Invitrogen). All cultures were performed at 37 °C in 5% CO₂. To withdraw cells from IL-3, they were washed three times and resuspended in complete medium lacking IL-3. To restimulate cells, they were pelleted and resuspended in complete medium containing IL-3. Cell size and concentration were determined using a Coulter Z2 particle analyzer or a hemocytometer. The PI3K inhibitor LY294002 (Cell Signaling) was diluted in Me₂SO to make a stock, and then diluted further in a 10% BSA solution at a 1:2 volume ratio. A total of 3.3 μl of [9,10-3H]palmitate and 6.7 μl of BSA were used per 1 ml of cell culture medium. Each sample used 0.5 × 10⁶ cells in 1 ml of medium supplemented with the [9,10-3H]palmitate-BSA mixture and cultured for 24 h in 24-well plates. After 24 h, supernatant was applied to ion-exchange columns (Dowex 1 X8 –200, Sigma), and tritiated water was recovered by eluting with 2.5 ml of H₂O. A 0.75-ml aliquot was then used for scintillation counting. For each data point, eight samples were prepared, four cultured without and four with 0.2 mM (+)-etomoxir sodium (Sigma), an inhibitor of mitochondrial long-chain fatty acid oxidation. To determine β-oxidation rate, a ranked pair analysis was used to determine the difference between oxidation counts in the absence and presence of etomoxir. The outlier among the four resulting values was discarded, and average and standard deviations were determined for the three remaining values.

To measure β-oxidation of endogenous lipid, 75 × 10⁶ cells were labeled in 150 ml of IL-3-containing medium supplemented with a [9,10-3H]palmitate-BSA mixture. This mixture was prepared by adding 40 μCi of [9,10-3H]palmitate to 1 ml of a 20 μM unlabeled palmitate stock in a solution of 10% essentially fatty acid-free BSA. This mixture was added to 150 ml of pre-warmed medium for culture. Cells were cultured for 2 days, and then harvested and washed three times in phosphate-buffered saline. The supernatant from the third wash contained negligible radioactivity. The labeled, washed cells were then cultured at a density of 0.25 × 10⁶ cells/ml in medium either containing or lacking IL-3 and containing or lacking etomoxir (0.2 mM). On subsequent days, aliquots of 0.5 ml were withdrawn from each flask, pelleted to remove cells, and applied to ion-exchange columns as above for determination of radioactivity. When cell density in IL-3-containing cultures exceeded 0.6 × 10⁶/ml, more medium was added to culture, and total dilution was used to correct oxidation counts.
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DNA Constructs—The murine Akt-1 containing a Src myristoylation sequence fused to the N terminus (myrAkt) was described elsewhere (33). The Akt1 open reading frame from this vector was subcloned into the expression vector, pBabe-IRES-GFP to create the vector pBabe-myrAkt-IRES-GFP. The CPT1A cDNA was amplified from murine liver cDNA and cloned into PCR2.1-TOPO (Invitrogen). The insert was then cloned as an EcoRI fragment into the retroviral construct MIGR1-GFP, creating MIGR1-GFP-CPT1A. Short hairpin RNA (shRNA) experiments used a published approach to express an shRNA under control of the human U6 RNA polymerase III promoter (34). Briefly, a PCR product was generated from the plasmid pE6-hU6 using primers that also contained a hairpin sequence against the CPT1A 19-mer 5′-GGCATATAACGCAGAGCATT-3′. This PCR product was cloned into pE6-TOPO, and then the promoter and hairpin were shuttled to a pBabe-puro-derived construct (34).

Cell Lines—The myrAkt-overexpressing clones and empty vector control cells for Figs. 2 and 3 were generated by electroporating IL-3-dependent bax−/−bak−/− cells with 8 μg of pBabe-myrAkt-IRES-GFP by Nucleofector Solution V (Amaza) using program T20. Five days later, green fluorescent protein (GFP)-positive cells were isolated by fluorescence-activated cell sorting to generate a bulk population of myrAkt-expressing cells. Stable clones were isolated by fluorescence-activated cell sorting with sorting into 96-well round bottom plates, and clones expressing high levels of myrAkt were identified by Western blotting. The shRNA clones and vector controls from Fig. 6 were generated in a similar fashion. Clones were screened by withdrawing cells from IL-3 and performing Western blots against CPT1A. To generate CPT1A-overexpressing and vector control IL-3-dependent cells (Fig. 7), we prepared retrovirus by co-transfecting 293T cells (Lipofectamine, Invitrogen) with MIGR1-GFP or MIGR1-GFP-CPT1A and a helper virus plasmid. Two days later, supernatants containing retroviral particles were collected. To infect IL-3-dependent cells, 3 × 10^6 cells were collected and resuspended in 1 ml of retrovirus-containing medium. Polybrene was added to a final concentration of 8 μg/ml, and the sample was centrifuged at 2200 rpm for 90 min. Cells were cultured at 37 °C for 5 h, then given fresh complete medium. On the third day after infection, GFP-positive cells were harvested by fluorescence-activated cell sorting.

Analysis of Microarray Data—Microarray data using cDNA samples from the IL-3-dependent cell line FL5.12 were reported previously (34). These experiments used cDNA samples prepared from triplicate cultures grown in the presence of IL-3 or 12 h after IL-3 withdrawal. cDNAs were hybridized to Affymetrix murine 11K oligonucleotide microarrays.

Western Blotting—Protein lysates were made by lysing cells in radioimmunoprecipitation buffer supplemented with complete protease inhibitor mixture (Roche Applied Science) and phosphatase inhibitors (Cocktails 1 and 2, Sigma). Protein was quantitated using BCA Protein Assay reagent (Pierce). Protein electrophoresis used NuPAGE 4–12% Bis-Tris gels (Invitrogen). The following primary antibodies were used: polyclonal total Akt and phospho-Akt (Sre-473, Cell Signaling Technology); monoclonal β-actin (Sigma); phospho-STAT5 A/B (Tyr-694/699, Upstate Biotech); and polyclonal CPT1A (a gift from Victor Zammit, Highland Research Institute, Scotland, UK). Horseradish peroxidase-conjugated secondary antibodies were anti-sheep (Upstate Biotech) anti-rabbit and anti-mouse (Amersham Biosciences). Proteins were detected with ECL Plus (Amersham Biosciences).

Quantitative Reverse Transcription-PCR—For quantitative reverse transcriptase-PCR (qPCR) analysis, total cellular RNA was prepared using Tri reagent (Sigma). Complementary DNA was generated from 1 μg of total RNA using SuperScript II reverse transcriptase (Invitrogen). Amplifications and data generation were performed using TaqMan probe sets, a 7900HT Sequence Detection System, and the SDS2.1 software (Applied Biosystems). Amplifications for β-actin and CPT1A were performed in triplicate, and β-actin mRNA abundance was used to normalize CPT1A mRNA abundance. Standard deviations were generated using the formula, \( [(RQ)\Delta C_{\text{S.D.}}]/\text{average } \Delta C \).

Lymphocyte Experiments—Adult female C57/B6 mice were sacrificed according to established mouse care protocols and in accordance with the Institutional Animal Care and Use Committee at The University of Pennsylvania. The spleen and lymph nodes (inguinal, axillary, and mesenteric) were harvested, crushed in Hanks’ basic salt solution, and washed once in phosphate-buffered saline lacking Ca^{2+} and Mg^{2+}, supplemented with 1% fetal calf serum. T lymphocytes were purified by negative selection using StemSep Mouse T-cell enrichment mixture (Stem Cell Technologies) and an AutoMACS separator (Miltenyi Biotec). After enrichment, T lymphocytes were either utilized directly for RNA, protein, and β-oxidation analysis, or were plated into wells pretreated with plate-bound anti-CD3 (Ebioscience) and anti-CD28 antibodies (BD Pharmingen) at 1 μg/ml each. Plated cells were cultured for 2 days, and then recombinant IL-2 was added to the medium at a concentration of 50 units/ml (PeproTech). After 2 more days, cells were harvested for RNA, protein, and β-oxidation analysis. To measure β-oxidation in T lymphocytes, 0.5 × 10^6 cells were plated into RPMI supplemented with [9,10-3H]palmitate-BSA as outlined above under “Lipid Metabolism Assays.” After a 4-h culture period, the medium was applied to ion-exchange resin as above.

RESULTS

Growth Factor Stimulation Simultaneously Increases Lipid Synthesis and Suppresses Fatty Acid Oxidation—The cytokine growth factor IL-3 stimulates phospholipid synthesis by activating the PI3K/Akt system (35). Free fatty acids used for phospholipid synthesis might come from the extracellular pool, from an intracellular pool generated by de novo fatty acid synthesis using glucose and other substrates, or from both. To compare the effects of IL-3 signaling on lipogenesis using these two fatty acid pools, we cultured IL-3-dependent cells with [14C]-labeled glucose or palmitate in the presence or absence of IL-3 and measured lipid synthesis. In the absence of IL-3, cells made a small amount of lipid using extracellular free fatty acids, but there was essentially no synthesis from glucose (Fig. 1A). By contrast, cells stimulated with IL-3 synthesized a larger amount of lipid using either substrate. IL-3 increased lipid synthesis from glucose by >100-fold (131.7 ± 28), whereas the increase in synthesis from palmitate was much less dramatic (3.2 ± 1).
Under all conditions tested, phospholipid was the predominant lipid species synthesized, accounting for >75% of recovered \(^{14}C\)-containing lipid.

A maximal rate of cell proliferation involves net synthesis of macromolecules and the avoidance of simultaneous synthesis and degradation of metabolic intermediates ("futile cycling"). Most cell types can degrade fatty acids through mitochondrial \(\beta\)-oxidation. We therefore tested whether growth factor stimulation affected the rate of \(\beta\)-oxidation. In absence of growth factor stimulation, IL-3-dependent cells exit the cell cycle and undergo progressive atrophy; re-introduction of IL-3 stimulates these quiescent cells to grow and re-enter the cell cycle (29, 36). Cells were cultured in the absence of IL-3 for 3 days and then re-stimulated by IL-3 addition. Cell growth, proliferation, de novo lipid synthesis, and fatty acid oxidation were examined periodically over the course of the experiment. Quiescent cells regained maximal size within 2 days of IL-3 stimulation and had begun to proliferate exponentially by the third day (data not shown). Lipid synthesis increased within the first day of IL-3 stimulation and was at maximal levels by the third day, coinciding with the onset of exponential proliferation (Fig. 1B). By contrast, the quiescent cells had a relatively high rate of \(\beta\)-oxidation of the fatty acid palmitate, and this was rapidly and persistently suppressed by introduction of the growth factor (Fig. 1B). By 72 h after IL-3 re-addition, the oxidation rate was suppressed to baseline levels.

**Growth Factor Suppression of \(\beta\)-Oxidation Requires PI3K and Akt Activity**—The effects of IL-3 on lipid synthesis have been reported to require activation of PI3K (35); therefore, we examined the role of this signaling pathway in suppressing \(\beta\)-oxidation. The PI3K inhibitor LY294002 increased \(\beta\)-oxidation rate in a dose-dependent fashion despite IL-3 stimulation (Fig. 2A). Because many metabolic effects of PI3K stimulation, including induction of lipid synthesis, are dependent on activation of Akt, we next examined the role of Akt in suppressing \(\beta\)-oxidation. To that end, a constitutively active allele of Akt was transfected stably into IL-3-dependent cells. Two independent clones expressing this construct maintained Akt phosphorylation (Fig. 2B) and continued to suppress \(\beta\)-oxidation (Fig. 2C) after IL-3 withdrawal for 1 day. Average \(\beta\)-oxidation activity and standard deviations are shown for three independent assays.
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withdrawal. Together, these results demonstrate that activation of the PI3K/Akt system by IL-3, in addition to enhancing lipid synthesis, also simultaneously suppresses β-oxidation of free fatty acids.

**Growth Factor Signaling Suppresses CPT1A Expression in Cell Lines and in Primary Lymphocytes—IL-3 and other growth factors elicit changes in cellular activity in part through effects on gene expression. We considered the possibility that the suppression of β-oxidation during IL-3 stimulation was due to decreased expression of one or more genes involved in this pathway. To test this hypothesis, we analyzed previously published microarray data to identify genes differentially regulated in the presence or absence of growth factor in the IL-3-dependent cell line FL5.12 (34). In this experiment, the expression of the liver isof orm of carnitine palmitoyltransferase I, CPT1A, was among the most highly regulated genes, exhibiting more than an 8-fold induction in cells withdrawn from IL-3 for 12 h (Table 1). No other enzyme involved in long chain β-oxidation was affected by a factor of more than 2-fold.

This result implied that CPT1A expression might be suppressed by IL-3 stimulation. To confirm this, we withdrew cells from IL-3 for 3 days, then re-stimulated and observed changes in the abundance of CPT1A mRNA and protein. Quantitative reverse transcription-PCR revealed a decrease in the abundance of CPT1A mRNA within 3 h of IL-3 stimulation (Fig. 3A). By Western blot, CPT1A protein abundance progressively decreased over the 72-h experimental period (Fig. 3B), the same time frame over which β-oxidation was suppressed. To determine the requirement for PI3K and Akt stimulation to suppress CPT1A, cells were stimulated with IL-3 and treated with the PI3K inhibitor LY294002. The drug abrogated the effect of IL-3 on CPT1A expression (Fig. 3C). Furthermore, cells with constitutive Akt activity failed to induce CPT1A expression after IL-3 withdrawal (Fig. 3D).

Next, the effects of growth factor stimulation on CPT1A expression and β-oxidation in primary lymphocytes were examined. We isolated T lymphocytes from adult female C57/Bl6 lymph nodes and spleen. These cells were either processed immediately, or stimulated in vitro with anti-CD3 and anti-CD28 in the presence of IL-2. Similar to IL-3 stimulation of hematopoietic progenitors, T lymphocyte stimulation induces Akt-dependent changes in metabolism, cell size, and proliferation (8). Stimulation of primary lymphocytes suppressed levels of CPT1A mRNA (Fig. 4A) and protein (Fig. 4B) and decreased the rate of β-oxidation (Fig. 4C).

**Growth Factor Withdrawal Increases CPT1A Expression and β-Oxidation of Both Exogenous and Endogenous Lipids—**When CPT1A expression and β-oxidation were examined after growth factor withdrawal, the abundance of CPT1A protein increased over the first 7 days (Fig. 5A). Oxidation of exogenous palmitate increased after IL-3 withdrawal, reached a maximum at 3 days, and thereafter declined (Fig. 5B).

In quiescent thymocytes, oxidation of unidentified, endogenous fuels has been found to contribute substantially to cellular bioenergetics (37). We therefore tested whether endogenous lipids might provide a substrate for β-oxidation in quiescent cells withdrawn from growth factor. First, we cultured cells in the presence of IL-3 and tritium-labeled palmitate for 48 h, over which time the fatty acid was used to generate cellular lipids as shown in Fig. 1. Then cells were washed to remove unincorporated label, and culture was resumed in the presence or absence of IL-3. Analysis of culture

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**TABLE 1**

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<th>Gene</th>
<th>Unigene symbol</th>
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<td>Cpt1A</td>
<td>+8.46</td>
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<tr>
<td>Mitochondrial carnitine/acycarnitine translocase</td>
<td>Slc25a20</td>
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<tr>
<td>Carnitine palmitoyltransferase 2</td>
<td>Cpt2</td>
<td>+1.38</td>
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<tr>
<td>Very long chain acyl-CoA dehydrogenase</td>
<td>Acadvl</td>
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<tr>
<td>Short chain acyl-CoA dehydrogenase</td>
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**FIGURE 3. IL-3 stimulation of PI3K/Akt suppresses expression of CPT1A.** A, cells were withdrawn from IL-3 for 3 days and then re-stimulated. Total RNA was harvested at the time points indicated and analyzed by quantitative reverse transcription-PCR (qPCR) for CPT1A abundance. Bars are average of three amplification reactions, with the CPT1A/β-actin ratio arbitrarily set to 1.0 for RNA collected from unstimulated cells (time 0). B, protein lysates were also collected from cells after a 3-day IL-3 withdrawal (time 0), or at various time points after re-stimulation (m, minutes; h, hours). Lysates were blotted and probed for CPT1A and for β-actin. As a control, protein was also collected from cells that had not been deprived of IL-3 (C), IL-3 withdrawal cells were re-stimulated with IL-3 in the absence or presence of the PI3K inhibitor LY294002 (LY). Phosphorylation of Akt at Ser-473 is normally observed within 15 min of IL-3 stimulation; LY abolished this phosphorylation, but not phosphorylation of STAT5, which is independent of PI3K activity (top four blots). After 48 h, protein was again collected and analyzed for CPT1A expression (bottom two blots). D, a vector clone and two clones overexpressing myrAkt were withdrawn from IL-3 for 1 day, and CPT1A abundance was compared with IL-3-stimulated cells by Western blot.

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**FIGURE 4. Effect of IL-3 withdrawal on expression of genes involved in β-oxidation.** Data are from previously reported microarray experiments with the IL-3-dependent cell line FL5.12 using Affymetrix murine 11K oligonucleotide microarrays (34). For each gene, -fold change represents the average of three independent experiments using RNA isolated in the presence of IL-3 or 12 h after the removal of IL-3. A positive -fold change reflects higher expression after IL-3 withdrawal.
medium each day revealed a continuous accumulation of $^3$H$_2$O, the end product of $^3$H$_2$O oxidation from labeled fatty acids (Fig. 5C). The rate of accumulation was much higher in the absence of IL-3, reflecting a significant and progressive induction of degradation and $^3$H$_2$O oxidation of endogenous lipids when cells were deprived of IL-3.

CPT1A Is Required to Maintain Viability in Growth Factor-deprived Cells—We next tested whether induction of CPT1A expression and $^3$H$_2$O oxidation were required for growth factor-deprived cells to maintain viability in culture. Clones stably expressing an shRNA against CPT1A were isolated. The CPT1A shRNA did not affect proliferation of IL-3-stimulated cells (Fig. S1), but it blunted CPT1A induction after IL-3 withdrawal (Fig. 6A, top). Two clones, one with intermediate CPT1A expression (shRNA-1) and another with nearly complete elimination of CPT1A expression (shRNA-2), were selected for further study. Both clones had reduced levels of $^3$H$_2$O oxidation induction after IL-3 withdrawal, suggesting that CPT1A expression was a limiting determinant of $^3$H$_2$O oxidation following growth factor withdrawal (Fig. 6A, bottom).

After IL-3 withdrawal, cell survival depends on maintaining bioenergetics through autophagy and residual levels of glycolysis (29). To test whether $^3$H$_2$O oxidation also contributed to cell survival during IL-3 withdrawal, clones expressing the CPT1A shRNA were withdrawn from IL-3 in medium containing reduced levels of glucose (0.3 mM). The CPT1A shRNA clones lost viability more rapidly than either a vector control clone or parental cells (Fig. 6B), suggesting that the cells’ ability to increase $^3$H$_2$O oxidation rate by expressing CPT1A is important for cell survival under these conditions. Consistent with this hypothesis, parental cells treated with the CPT1 inhibitor eoto-

FIGURE 4. In vitro co-stimulation of primary T lymphocytes suppresses CPT1A expression and $^3$H$_2$O oxidation. T lymphocytes were harvested from adult C57Bl6 mice. Half the cells were used to isolate RNA and protein, and the other half were stimulated with $\alpha$-CD3/CD28 antibodies and IL-2. Abundance of CPT1A mRNA (A) and protein (B) were determined by qPCR and Western blot, respectively. The qPCR data are the average relative quantities, and standard deviations were determined from three amplifications. C, palmitate $^3$H$_2$O oxidation was compared between naive T lymphocytes immediately after harvest, and cells that had been stimulated with $\alpha$-CD3/CD28 and IL-2. Average oxidation rates and standard deviations from three assays are shown.

FIGURE 5. IL-3 withdrawal increases $^3$H$_2$O oxidation of exogenous and endogenous lipids. IL-3-dependent cells were withdrawn from IL-3, and CPT1A protein abundance (A) and rate of $^3$H$_2$O oxidation (B) were determined at the indicated time points. Each oxidation measurement reflects the average and standard deviation generated from three independent assays. C, cells were cultured with [9,10-$^3$H]palmitate in the presence of IL-3 for 48 h, then washed to remove unincorporated palmitate and cultured in the presence or absence of IL-3. The cumulative amount of $^3$H$_2$O oxidation of labeled fatty acids generated from endogenous lipid was determined each day. Each data point reflects the average and standard deviation of three measurements.
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FIGURE 6. Increased CPT1A expression and β-oxidation are required for survival during withdrawal of glucose and growth factors. A, IL-3-dependent clones expressing an shRNA against CPT1A (shRNA-1 and -2) had decreased abundance of CPT1A (top) and β-oxidation (bottom) when withdrawn from IL-3. Parental cells and a vector control clone (vec-1) are shown for comparison. Average β-oxidation activity and standard deviations are shown for three independent assays. B, parental IL-3-dependent cells, a vector control clone, and two shRNA clones were cultured in medium containing low glucose (0.3 mM) and no IL-3. Viability was determined by exclusion of propidium iodide at the indicated times. Each data point reflects the average and standard deviation for three cultures processed in parallel.

FIGURE 7. Constitutive CPT1A expression increases β-oxidation rate and decreases lipid synthesis and cell proliferation. A, IL-3-dependent cells were infected with a retroviral vector containing the mouse CPT1A cDNA (MIGR1-CPT1A). Abundance of CPT1A protein (top) and rate of β-oxidation (bottom) were determined in the presence of IL-3 and compared with cells infected with a control retrovirus (MIGR1-vec). B, vector control and CPT1A-overexpressing cells were assayed for their ability to convert [14C]glucose into lipids during IL-3 stimulation. Cells were cultured in the presence of [14C]glucose for 24 h, then lipids were harvested and analyzed by scintillation counting. Bars represent the average and standard deviations of three measurements. C, cells infected with MIGR1-vec (open circles) or MIGR1-CPT1A (filled circles) were analyzed for proliferation rate soon after retroviral infection. Cumulative population doublings were calculated over 6 days of culture.

Discussion

In mammalian cells, unlike unicellular organisms, it has been proposed that metabolism is regulated by growth factor signaling rather than by the availability of extracellular nutrients. This hypothesis is based on the observations that growth factors stimulate metabolic activities associated with cell growth and proliferation, including glucose uptake, glycolysis, and lipid synthesis, and that in the absence of growth factor signaling, nutrient utilization is diminished (8, 29). We reasoned that growth factor signaling might also suppress catabolism of cellular constituents and metabolic intermediates to maximize macromolecular synthesis during cell proliferation. The data show that growth factor stimulation suppresses degradation of cellular lipids and β-oxidation of fatty acids, whereas growth factor deprivation or interruption of PI3K signaling forces cells into a state of lipid catabolism despite the continued abundance of extracellular nutrients. The data strengthen the hypothesis that a major role of growth factor signaling is to instruct cells to engage in metabolic activities that support growth.

PI3K/Akt signaling executes many of the anabolic activities of growth factor-stimulated cells, including lipid and protein synthesis. Its involvement in suppressing catabolic metabolism at the cellular level is now also becoming clear. Cytokine stimulation of PI3K/Akt enhances lipid synthesis in IL-3-dependent cells (35), and the concomitant, inverse effect on β-oxidation that we observed is predicted to reduce futile cycling and maximize the fatty acid pool available for lipid synthesis. The role of PI3K/Akt signaling in protein metabolism is similar. During cell growth, PI3K/Akt-mediated activation of mammalian target of rapamycin activates protein translation and simultaneously suppresses autophagic degradation of proteins (38), changes that together would maximize protein synthesis. Therefore, the role of PI3K/Akt in cellular metabolism is more than merely to increase the rate of biosynthesis. It also suppresses degradation of at least two major classes of cellular macromolecules.

The rapid decline in the abundance of the CPT1A mRNA after IL-3 stimulation (Fig. 3A) may involve an abrogation of
ongoing transcription, an enhancement of mRNA degradation, or a combination. This result demonstrates that the effect of PI3K/Akt stimulation on CPT1A expression is not occurring at the translational or post-translational level. This is an important distinction, because recent work has implicated translational effects as the major mechanism by which PI3K/Akt impacts gene expression in the acute phase (39). The present work suggests that CPT1A can be regulated by a different mechanism in which the primary effect is on mRNA abundance.

It was surprising to us that IL-3 signaling decreased the β-oxidation rate through PI3K-mediated suppression of CPT1A expression, because the predominant mechanism of β-oxidation control in muscle and liver does not require changes in gene expression. In those tissues, β-oxidation is dynamically regulated in response to transient, cyclical stimuli (feeding/fasting and rest/exercise) that periodically change the reliance on fatty acids for fuel. In the short term, β-oxidation rate is controlled by allosteric inhibition of CPT I by malonyl-CoA, a cellular marker of nutritional abundance that is produced by the enzyme acetyl-CoA carboxylase (ACC), the committed step in fatty acid synthesis (26, 40). In nutritionally and energetically replete cells, malonyl-CoA inhibits CPT I, and this can be reversed almost immediately if the need for β-oxidation increases. In hematopoietic cells, by contrast, while malonyl-CoA might impact β-oxidation in the short transition between the onset of stimulation and disappearance of CPT1A protein, the major regulatory mechanism is at the level of gene expression. We hypothesize that the regulation of CPT1A expression in hematopoietic cells reflects the fact that such cells do not typically require short-term suppression of β-oxidation. Rather, in these cells, stimulation with growth factors or antigens signals the need for a commitment to growth lasting hours to days and culminating in cell proliferation. Based on our data, cells respond by reducing CPT1A expression, stably suppressing β-oxidation throughout the proliferative response. In this regard, it is also noteworthy that activation of human and murine T lymphocytes stimulated the AMP-activated protein kinase, a key regulator of cellular bioenergetic homeostasis that can also enhance β-oxidation in muscle (41, 42). The suppression of CPT1A expression that we observed after antigen-induced stimulation of T lymphocytes would explain how these cells undergo simultaneous activation of AMP-activated protein kinase and inhibition of β-oxidation.

It was also surprising that CPT1A overexpression was sufficient to increase β-oxidation in the presence of IL-3 (Fig. 7A), considering that IL-3 stimulation increases lipogenesis and therefore malonyl-CoA production. In the presence of ongoing lipogenesis, we anticipated that the β-oxidation rate would remain low. However, malonyl-CoA sensitivity is determined in part by which isoform of ACC is present in the cell. ACC1 is widely expressed, located in the cytosol, and generates malonyl-CoA used in fatty acid synthesis (43). ACC2, on the other hand, is restricted to liver and muscle (44), targeted to the outer mitochondrial membrane, and has been suggested to provide a localized malonyl-CoA pool that modulates CPT I activity (45). Hepatocytes lacking ACC2 exhibit constitutive β-oxidation despite the presence of ACC1 (46, 47). Most cell types do not express ACC2, and we found no evidence for expression of this protein in IL-3-dependent cells (data not shown). In absence of ACC2, the malonyl-CoA produced during lipogenesis appears not to be sufficient to inhibit β-oxidation if CPT1A is expressed. The data suggest that proliferative cells, unlike hepatocytes and myocytes, solve this problem by growth factor-initiated suppression of CPT1A, and failing to do so negatively impacts cell growth.

Hematopoietic cells have not traditionally been thought to regulate β-oxidation, but a few other studies have provided evidence suggesting that this is the case. CPT1A expression was enhanced during death induced by cytokine withdrawal in several hematopoietic cell lines, and therefore β-oxidation was proposed to function in apoptosis (48, 49). More recently, differential regulation of CPT1A expression and β-oxidation was identified as a marker discriminating between Th1- and Th2-mediated macrophage activation (50). In the classic inflammatory pathway induced by Th1 cytokines, expression of CPT1A and other β-oxidation genes was suppressed. In contrast, macrophage activation by Th2 cytokines attenuated the inflammatory response while enhancing expression of β-oxidation genes and β-oxidation activity. Similar to that study, we also found that cytokines regulate β-oxidation and that this regulation can have functional consequences in both stimulated and unstimulated cells.

The increased CPT1A abundance and β-oxidation following cytokine withdrawal have implications for the bioenergetic strategy of quiescent cells. First, they imply a greater role for β-oxidation during quiescence than during stimulation, which may be important given the gradual reduction in glycolysis during cytokine withdrawal (29). The premature loss of viability in cells expressing the CPT1A shRNA is consistent with this hypothesis (Fig. 6B). Second, in addition to their ability to oxidize fatty acids from extracellular sources, quiescent cells also oxidize fatty acids obtained from endogenous lipids (Fig. 5C), implying that lipid turnover during cellular atrophy provides metabolically useful substrates. Because cells undergo autophagy during IL-3 withdrawal, we speculate that fatty acids are generated during autophagic degradation of the lipid components of cellular organelles. In support of this hypothesis, earlier experiments on thymocyte metabolism suggested that an unidentified “endogenous fuel” accounted for ~60% of respiration and ATP turnover in the quiescent state (37). Our data suggest that fatty acid oxidation explains this phenomenon.

In cancer, tumor cells acquire increased metabolic autonomy and decreased dependence on growth factor signaling for the import and utilization of nutrients. Activation of PI3K/Akt signaling is one of the most common genetic mechanisms of human cancer (51), and tumor cells with constitutive activity of the pathway develop a high rate of glucose import, aerobic glycolysis, and lipid synthesis (18, 32, 52). In addition, PI3K/Akt transformation renders cells absolutely dependent on glucose for survival because of a reduced capacity to augment β-oxidation during glucose withdrawal; this glucose “addiction” can be exploited to kill cells in vitro (32, 52). Our data suggest that PI3K-mediated suppression of CPT1A expression and β-oxidation contributes to this effect and, therefore, represents another core metabolic pathway whose regulation is fundamentally dis-
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turbed during the metabolic transformation of PI3K/Akt-driven tumorigenesis.

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REFERENCES

Phosphatidylinositol 3-Kinase-dependent Modulation of Carnitine Palmitoyltransferase 1A Expression Regulates Lipid Metabolism during Hematopoietic Cell Growth
Ralph J. DeBerardinis, Julian J. Lum and Craig B. Thompson

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