Aberrant LPL Expression, Driven by STAT3, Mediates Free Fatty Acid Metabolism in CLL Cells

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Abstract

While reviewing chronic lymphocytic leukemia (CLL) bone marrow slides, we identified cytoplasmic lipid vacuoles in CLL cells but not in normal B cells. Because lipoprotein lipase (LPL), which catalyzes hydrolysis of triglycerides into free fatty acids (FFA), is aberrantly expressed in CLL, we investigated whether LPL regulates the oxidative metabolic capacity of CLL cells. We found that unlike normal B cells, CLL cells metabolize FFAs. Because STAT3 is constitutively activated in CLL cells and because we identified putative STAT3 binding sites in the LPL promoter, we sought to determine whether STAT3 drives the aberrant expression of LPL. Transfection of luciferase reporter gene constructs driven by LPL promoter fragments into MM1 cells revealed that STAT3 activates the LPL promoter. In addition, chromatin immunoprecipitation confirmed that STAT3 binds to the LPL promoter. Furthermore, transfection of CLL cells with STAT3-shRNA downregulated LPL transcripts and protein levels, confirming that STAT3 activates the LPL gene. Finally, transfection of CLL cells with LPL-siRNAs decreased the capacity of CLL cells to oxidize FFAs and reduced cell viability.

Implications: Our study suggests that CLL cells adopt their metabolism to oxidize FFA. Activated STAT3 induces LPL, which catalyzes the hydrolysis of triglycerides into FFA. Therefore, inhibition of STAT3 is likely to prevent the capacity of CLL cells to utilize FFA.

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Introduction

Chronic lymphocytic leukemia (CLL) is characterized by the gradual accumulation of mature-appearing lymphocytes (1) whose gene expression profile and expression of the cell surface CD27 antigen resemble typical features of memory B cells (2,3). Normally, memory B cells are quiescent, and extracellular stimuli, such as the CD40 ligand, are required to induce their proliferation (4). Unlike memory B cells, approximately 1% of CLL cells proliferate daily (5). However, what energy source CLL cells use and which metabolic pathways they recruit to provide the energy needed for survival and proliferation are not known.

Lipoprotein lipase (LPL), commonly expressed in adipocytes and muscle cells, plays a central role in lipid metabolism (6). LPL catalyzes the hydrolysis of triglycerides into free fatty acids (FFA) and increases the cellular uptake of lipoproteins in a noncatalytic manner (7).

LPL transcripts were found in CLL cells but not in normal B lymphocytes (3, 8), and high levels of LPL were detected on the cell surfaces and in the cytosol of CLL cells (9). Remarkably, increased levels of LPL-mRNA were correlated with unmutated immunoglobulin heavy-chain variable region genes, aggressive disease, and an unfavorable prognosis (3, 8–11). However, what activates LPL in CLL has not been deciphered.

STAT3 is a latent cytoplasmic transcription factor that relays cytokine and growth factor signals from the cell membrane to the nucleus (12). In CLL, STAT3 is constitutively phosphorylated on serine 727 residues. Serine pSTAT3 migrates to the nucleus, binds to DNA, activates transcription, and provides CLL cells with a survival advantage (13, 14).

In several human tumors, signal transduction pathways regulate metabolic pathways, and in some of those tumors, lipid metabolism is altered (15). Because STAT3 was found to modulate lipid synthesis and modify the expression of genes that regulate cellular metabolism (16), and because sequence analysis revealed that the LPL promoter harbors γ-interferon activation sequence (GAS)—like elements, known to bind STAT3, we sought to determine whether STAT3 activates the transcription of LPL.

Here, we show that the aberrant expression of LPL is driven by STAT3. Unlike normal B cells, the cytoplasm of CLL cells contains lipid-filled vacuoles, and the cells utilize fat as an energy source in an LPL-dependent manner.

Materials and Methods

Fractionation of CLL cells and normal B cells

Peripheral blood cells were obtained from previously untreated patients with CLL who were followed at The University of Texas MD Anderson Cancer Center Leukemia Clinic after we received...
Institutional Review Board approval and written informed consent from the patients (Supplementary Table S1). To isolate low-density cells, the patients’ peripheral blood cells were fractionated using Ficoll–Hypaque 1077 (Sigma-Aldrich). More than 90% of the peripheral blood lymphocytes obtained from these patients were CD19+/CD5−, as assessed by flow cytometry (Becton, Dickinson and Company). Peripheral blood samples from healthy donors were obtained from the Central Blood Bank as buffy coats. After Ficoll–Hypaque fractionation, the donors’ B cells were isolated using Miltenyi CD19-coated beads according to the manufacturer’s instructions (Miltenyi Biotec).

**Cell culture**

The fractionated CLL cells were maintained in DMEM (Sigma-Aldrich) supplemented with 10% FBS (HyClone). Cells from the human multiple myeloma line MM1 were obtained from the ATCC. MM1 cells were maintained in RPMI 1640 (Sigma-Aldrich) supplemented with 10% FBS in a humidified atmosphere of 5% CO2 at 37°C. The human renal epithelial carcinoma 293T cells were grown in DMEM (Sigma-Aldrich) supplemented with 10% FCS (HyClone). Human umbilical vein endothelial cells (HUVEC) were maintained in vascular endothelial medium (both from Lonza).

**Oil Red O staining of bone marrow aspirates from CLL patients**

Slides of bone marrow aspirates from the patients with CLL were placed in absolute propylene glycol for 2 minutes and rinsed in distilled water, and mounted in a warmed glycerin jelly stainer, and examined using a JEM 1010 transmission electron microscope (JEOL USA, Inc.). The slides were viewed using an Olympus FluoView 500 Laser Scanning Confocal Microscope (Olympus America), and images were analyzed using the FluoView software (Olympus America).

**Western blot analysis**

Western blot analysis was performed as previously described (18). Briefly, cell lysates were assayed for their protein concentrations using the bicinchoninic acid protein assay reagent (Pierce Chemical). Each set of paired lysate was adjusted for the same protein concentration. A lysate of CLL cell extract was mixed with 4x Laemmli sample buffer and was then denatured by boiling for 5 minutes. Forty micrograms of lysates were dissolved separated using 8% SDS–PAGE and then transferred to a nitrocellulose. The transfer was done overnight at 30 V in a cooled (4°C) reservoir. The nitrocellulose membrane was then placed in a Ponceau S staining to verify equal loading of protein. The membranes were blocked with 5% dried milk dissolved in 50 mL of PBS. After blocking, the membrane was incubated with the following primary antibodies: monoclonal mouse anti-human STAT3 (BD Biosciences; Cat# 610190; in a dilution of 1:2 × 10³), monoclonal rabbit anti-human Tyr705 STAT3 antibodies (Cat# 9131; Cell Signaling; in a 1:10³ dilution), monoclonal mouse anti-human LPL (Abcam; Cat# 21356; in a dilution of 1:10³), and mouse anti-human β-actin (Sigma-Aldrich). After incubation with horseradish peroxidase–conjugated secondary antibodies (GE Healthcare) for 1 hour, blots were visualized with an enhanced chemiluminescence detection system (GE Healthcare).

Densitometry analysis was performed using an Epson Expression 1680 scanner (Epson America, Inc.). Densitometry values were normalized by dividing the numerical value of each sample signal by the numerical value of the signal from the corresponding levels of each sample’s density by the density of the corresponding β-actin protein, used as a loading control.

**Measurement of cellular O2 consumption**

Because fatty acid metabolism increases O2 consumption, palmitic acid, and oleic acid, utilization was assessed by measuring the level of dissolved O2 (dO2) using the SevenGo pro Dissolved Oxygen Meter (Mettler Toledo).

Preliminary experiments designed to test FFA consumption that used palmitic acid or oleic acid dissolved in ethanol determined that the O2 consumption with 80 mmol/L palmitic acid and 2 mmol/L oleic acid each (both from Sigma-Aldrich) is maximal, and therefore, we used these concentrations in the following experiments. In these experiments, we also found that when used in combination palmitic acid and oleic acid increase O2 consumption more than each of these components separately (data not shown).

In each experiment, we used CLL cells, normal B cells, or HUVECs at a concentration of 2 to 3 × 10³ cells/mL. The cells were incubated with a minimum essential medium (MEM) with Hank’s salts and l-glutamine (Life Technologies) or with PBS medium (Invitrogen) for 48 to 72 hours in tightly sealed T25 tissue culture flasks (Corning) at 37°C in the presence or absence of palmitic acid or oleic acid. In control experiments, CLL cells were incubated in PBS with or without ethanol. The O2 meter probe was placed in the flask, and the reading allowed stabilizing. Then, the dO2 level was recorded. The probe was cleaned before it.
was reused. Measurements of dO2 were repeated at least three times for every data point. We used the Student t test when one experimental condition was compared to nontreated (controls) and one-way ANOVA when 2 experimental conditions were compared with controls. Statistical analyses were performed using GraphPad version 5.

Transfection of CLL cells with LPL siRNA

Five microliters of siPORT NeoFX agent and 50 pmol of either siRNA-targeting LPL (Applied Biosystems) or the FAM-labeled siRNA targeting the human glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Life Science Technologies) were each diluted in 50 μL of OPTI-MEM I and then mixed together and incubated at room temperature for 10 minutes. A total of 5 × 10⁶ cells suspended in 0.2 mL of OPTI-MEM I medium containing the siRNA and transfection agent were incubated at room temperature. After 1 hour of incubation, transfections were performed by electroporation (Bio-Rad Laboratories), and then the cells were cultured in complete RPMI 1640 medium. Transfection efficiency was calculated on the basis of the GFP-conjugated siRNA measured by flow cytometry (Becton, Dickinson and Company).

Transfection of MM1 cells with LPL promoter fragments and luciferase assay

LPL promoter fragments were transfected into MM1 cells by electroporation as previously described (19, 20). Each construct included the luciferase reporter gene and the fragment either 143 bp upstream of the transcription start site (TSS) of the LPL gene, a region that includes one putative STAT3 binding site, or 333 bp upstream of the LPL gene TSS, a region that includes two putative STAT3 binding sites. The luciferase activity of unstimulated or IL6-stimulated MM1 cells was assessed 48 hours after transfection using the Dual-Luciferase Reporter Assay.
System (Promega) and the SIRIUS luminometer V3.1 Berthold Detection Systems (Titertek-Berthold). The luciferase activity of each of the human LPL promoter constructs was determined by calculating the constructs’ luciferase activity relative to the activity of the Renilla luciferase produced by the pRL-SV40 control vector.

Chromatin immunoprecipitation assay

The chromatin immunoprecipitation (ChIP) assay was performed according to manufacturer’s of the SimpleChIP Enzymatic Chromatin IP Kit (Cell Signaling Technology) as previously described (20). The primers to amplify the human LPL promoter were F: AGC AAT GAG GTA TGT GTG TAG and R: CTA CAT CAT TAT CAG GGT TAC, which generate a 144-bp product that covers the promoter region flanking 1 to 144 bp upstream of LPL; F: GAG ATT GAA ACT GGT TAC, which generate a 144-bp product that covers the promoter region flanking 1 to 144 bp upstream of LPL; and F: ATT TCT TCA GCA GGG TTT GCC and R: AAA CTA TGG GAT TCC TAG GGG, which generate a 111-bp product that covers the promoter region flanking 250 to 333 bp upstream of LPL.

Infection of CLL cells with GFP-conjugated lentiviral STAT3 shRNA

The supernatant of 293T cells, cotransfected with GFP-lentivirus STAT3-shRNA (shRNA) or GFP-lentivirus empty vector and the packaging vectors pCMVdeltaR8.2, was used to infect CLL cells with STAT3-shRNA or the empty vector as previously described (14). Briefly, CLL cells (5 × 10^6/mL) were incubated in 6-well plates (Becton Dickinson) in 2 mL DMEM supplemented with 10% FCS and transfected with 100 μL of viral supernatant. Polybrene (10 ng/mL) was added to the viral supernatant at a ratio of 1:1,000 (v/v). Transfection efficiency was measured after 48 hours and was found to range between 30% and 60% [calculated on the basis of the ratio of propidium iodide (PI)–negative/GFP-positive cells].

RNA purification and quantitative reverse transcription PCR

RNA was isolated using an RNasey purification procedure (QIAGEN Inc.). RNA quality and concentration were analyzed with a NanoDrop spectrophotometer (ND-1000; NanoDrop Products). Five hundred nanograms of total RNA was used in one-step quantitative reverse transcription PCR (qRT-PCR) with the sequence detection system ABI Prism 7700 using TaqMan gene expression assays (all from Applied Biosystems) for STAT3, ROR1, c-Myc, cyclin D1, p21, VEGF-c, RPL30, and LPL according to the manufacturer’s instructions. Samples were run in triplicate, and relative quantification was performed using the comparative CT method (20).

Apoptosis assay

The rate of cellular apoptosis was analyzed using double staining with a Cy5-conjugated Annexin V kit and PI (BD Biosciences) according to the manufacturer’s instructions. Briefly, 1 × 10^6 cells were washed once with PBS and resuspended in 200 μL binding buffer with 0.5 μg/mL Annexin V–Cy5 and 2 μg/mL PI. After incubation for 10 minutes in the dark at room temperature, the samples were analyzed on a FACS Calibur flow cytometer (BD Biosciences). Cell viability was calculated as the percentage of Annexin V–positive cells.

Results

Lipid-like vacuoles were detected in CLL cells but not in normal B cells

In reviewing bone marrow biopsies of patients with CLL, we identified clear-appearing vacuoles in the cytoplasm of CLL but not normal bone marrow cells. Because clear-appearing inclusion vacuoles are typically those of stored lipids, we stained CLL bone marrow smears from two CLL patients with Oil Red O. As shown in Fig. 1A, Oil Red O staining confirmed that lipid deposits are present in CLL bone marrow cells (Fig. 1A). To confirm that these lipid deposits were present within the cells, we used TEM and found that the lipid vacuoles visualized by TEM were present in the cytoplasm of 95% of CLL peripheral blood cells (Fig. 1B) but not in normal B cells obtained from healthy individuals (Fig. 1C).

LPL was detected on the surface and in the cytosol of CLL cells

Several reports have suggested that CLL cells, like other lipid vacuole–containing cells (e.g., adipocytes and myocytes; refs. 21–23), express LPL (3, 8, 24). To confirm this observation, we performed Western blot analysis on low-density peripheral blood cells from 5 CLL patients. As shown in Fig. 2A, we detected LPL protein in all five CLL patients’ samples but not in normal B cells. Then, using confocal microscopy, we confirmed this observation and found that LPL was present on the surfaces and in the cytoplasm of CLL cells (Fig. 2B).
Aberrant, STAT3-Driven LPL Mediates CLL Metabolism

**A**

![Graphs showing relative O₂ concentration for different treatments.](image)

**B**

![Graphs showing relative O₂ concentration for different treatments.](image)

**C**

![Fluorescence images showing Annexin V and PI staining for different treatments.](image)

**D**

![Bar graph showing delta apoptosis rate for GAPDH and LPL-siRNA.](image)

**E**

![Western blots showing LPL and 18S levels.](image)
FFAs increase the metabolic rate of CLL cells

The presence of lipid vacuoles in the cytoplasm of CLL cells suggested that, like adipocytes and myocytes, CLL cells utilize lipids as a cellular energy source. Active metabolism increases O₂ consumption and, as a result, decreases the levels of dO₂ in the cells' culture medium. Therefore, to estimate levels of CLL cell metabolism, we measured the levels of dO₂ in the medium of the CLL cells incubated in the presence or absence of palmitic acid or oleic acid. After 48 hours of incubation with or without glucose-free media, the levels of dO₂ were significantly lower in the presence of palmitic acid or oleic acid than in the absence of either FFA (Fig. 3A). In contrast, incubation of normal B cells with palmitic acid or oleic acid did not change the dO₂ levels in the culture media of normal B cells (Fig. 3A) or HUVEC cells (data not shown), suggesting that CLL cells, but not normal B cells, metabolize FFAs. To determine whether the decrease in dO₂ resulted from increased cellular metabolism, we performed an additional experiment in which we measured dO₂ in the presence or absence of CLL cells. An insignificant decrease in dO₂ was observed in the absence of cells. However, the addition of CLL cells significantly decreased dO₂ and the addition of FFA induced the most significant decrease in dO₂ levels, suggesting that increased cellular metabolism induced a major reduction in dO₂ levels (Fig. 3B).

LPL provides CLL cells with survival advantage

Although LPL is universally overexpressed in CLL cells, LPL's mRNA and protein levels were reported to be higher in high-risk CLL(9). To test whether LPL provides CLL cells with survival advantage, we transfected CLL cells with LPL-shRNA. While a considerable overlap in cell viability between cells treated with LPL-shRNA and controls was observed, LPL knockdown significantly reduced CLL cell viability (Fig. 3C and D) and abrogated dO₂ consumption in the presence of palmitic acid, suggesting that LPL-shRNA reduced the capacity of CLL cells to oxidize FFA (Fig. 3E).

STAT3 activates the LPL promoter in MM1 cells

Because STAT3 is constitutively phosphorylated (14) and LPL is aberrantly expressed in CLL cells (3, 8), we wondered whether STAT3 activates the transcription of LPL. Using the TFSEARCH database (25), we identified two GAS-like elements that are known putative STAT3 binding sites (26) within 400 bp upstream of the LPL TSS. To determine whether STAT3 activates the LPL promoter, we used MM1 cells. IL6 induces the phosphorylation of STAT3 in various cell types, including MM1 cells (14, 27, 28), thus providing us with a pSTAT3-inducible system. Incubation of MM1 cells with IL6 induced STAT3 phosphorylation and upregulated LPL protein levels in a dose- and time-dependent manner (Fig. 4A).

To assess the capability of each putative STAT3 binding site to activate the LPL promoter activity, we transfected MM1 cells with the luciferase reporter gene driven by fragments of the LPL promoter (Fig. 4B, top). As shown in the lower plot of Fig. 4B, the promoter activity was detected only in MM1 cells transfected with the 333-bp fragment and not in those transfected with the 143-bp fragment, suggesting that the GAS-like element located between bp –280 and bp –294 but not the one between bp –86 and bp –95 upstream of the TSS is an active STAT3 binding site. Moreover, when MM1 cells transfected with the 333-bp promoter fragment were incubated with IL6, the promoter activity was markedly increased (Fig. 4B).

To decipher this observation, we used a ChIP assay. As shown in Fig. 4C, only primer 2, corresponding to the –333 bp to –250 bp sequence, but not primer 1 (downstream of primer 2) or primer 3 (upstream of primer 2), amplified DNA that coimmunoprecipitated with anti-STAT3 antibodies (Fig. 4C). To determine whether pSTAT3-LPL promoter binding activates the transcription of LPL, we transfected MM1 cells with STAT3 siRNA, incubated them with IL6 for 2 hours, and assessed LPL and various other STAT3-regulated genes' mRNA levels using qRT-PCR. As shown in Fig. 4D, STAT3 siRNA downregulated mRNA levels of LPL and the STAT3-regulated genes STAT3, Bcl2, c-Myc, and p21/WAF1. In addition, transfection of MM1 cells with STAT3-siRNA, but not with scrambled siRNA or GAPDH, downregulated STAT3 protein levels by 60% and LPL protein levels by 70% (Fig. 4E), suggesting that STAT3 binds to the LPL promoter, activates the LPL gene, and induces the production of LPL protein in MM1 cells.

STAT3 activates the LPL promoter in CLL cells

After establishing that STAT3 activates the LPL promoter in MM1 cells, we sought to determine whether STAT3 activates the
Aberrant, STAT3-Driven LPL Mediates CLL Metabolism

A

LPL (53 Kd)

Fold

pSTAT3 (Tyr705)

Fold

STAT3

Time (h)

IL6 (ng/mL)

B

-298 CTGAGTTTCTTGAAAATTGG -278

-95 TTCTCAAGAA -86

-333 bp

Luc

-143 bp

Luc

Relative luciferase activity (RLU/s X1,000)

C

STAT3 Abs.

Input Cont. IL6

Primer 1 (-674 to -563 bp)

Primer 2 (-333 to -250 bp)

Primer 3 (-144 to +1 bp)

D

CTRL STAT3 Bcl2 c-Myc p21 LPL

Fold change

E

STAT3

Fold

LPL

Fold

β-Actin

HEA Control Scrambled RNA STAT3 RNA GAPDH

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transcription of LPL in CLL cells. To that end, we performed ChIP. As shown in Fig. 5A, we found that anti-STAT3 antibodies coimmunoprecipitated DNA of the LPL promoter and of the STAT3-regulated genes STAT3, c-Myc, p21, VEGF-c, and ROR1. As in MM1 cells, primers of the LPL promoter binding site 2, but not site 1 or 3, amplified the STAT3 coimmunoprecipitated DNA (Fig. 5A, right), suggesting that the GAS-like element 280 bp upstream of the LPL coding region is an active STAT3 binding site in CLL.

We then sought to determine whether STAT3 activates LPL in CLL cells. We transfected CLL cells with a lentiviral STAT3 shRNA vector. Compared with cells transfected with empty vector or STAT3-shRNA or with an empty vector, STAT3 shRNA downregulated STAT3 and LPL protein levels by 80%.

Figure 4.
STAT3 activates the LPL promoter in MM1 cells. A, detection of pSTAT3 and LPL in IL6-stimulated MM1 cells. MM1 cells were incubated with increasing concentrations of IL6 (0 to 20 ng/mL) for 2 hours (left), and with 10 ng/mL of IL6 for 0 to 4 hours (right). Cell lysates underwent Western blot analysis with anti-tyrosine pSTAT3, anti-STAT3, and anti-LPL antibodies. HeLa cells incubated with IL6 for 4 hours were used as positive controls in each panel. Incubation with IL6 increased the levels of tyrosine pSTAT3 and LPL in a dose- and time-dependent manner. B, on the basis of the presence and locations of GAS-like elements in the LPL promoter (top), we transfected MM1 cells with truncated forms of the LPL promoter and the luciferase reporter gene (left bottom). In the right bottom plot, the horizontal bars show the mean ± SEM for luciferase activity levels of transfected MM1 cells incubated without or with 20 ng/mL IL6. C, Western blot analysis of CLL cells transfected with STAT3 shRNA or empty vector showed that compared with an empty vector, STAT3 shRNA downregulated STAT3 and LPL protein levels by 80%.

Figure 5.
STAT3 activates the LPL promoter in CLL cells. A, ChIP assay. CLL cell protein extract was incubated without or with anti-STAT3 antibodies, and DNA was extracted from chromatin fragments. As shown in the left plot, anti-STAT3 antibodies coimmunoprecipitated DNA of the STAT3 target genes c-Myc, p21, STAT3, VEGF-c, and ROR1 but not of the ribosomal RPL30 gene (used as negative control). As in Fig. 4C, “Input” denotes DNA extracted from nonimmunoprecipitated CLL cell chromatin fragments (negative control) IgG is the isotype of the anti-STAT3 antibodies. The right top panel depicts ChIP of CLL cells. As shown, STAT3 coimmunoprecipitated DNA that was amplified with primers designed to amplify site 2 but not primers designed to amplify site 1 or site 3 of the LPL promoter regions. The right bottom panel depicts results of two separate experiments analyzed using qRT-PCR. Similar to the results depicted in the right top panel, STAT3 coimmunoprecipitated DNA was significantly amplified with primer 2. B, CLL cells were transfected with STAT3-shRNA or with an empty vector. Compared with cells transfected with empty vector (CTRL), the cells that were transfected with STAT3-siRNA expressed significantly lower levels of STAT3-regulated genes including LPL. C, Western blot analysis of CLL cells transfected with STAT3-shRNA or empty vector showed that compared with an empty vector, STAT3 shRNA downregulated STAT3 and LPL protein levels by 80%.
(transfection efficiency, 50%) or with an empty vector and quantified LPL and STAT3-regulated gene miRNA levels by using relative qRT-PCR. As shown in Fig. 5B, transfection with STAT3 shRNA reduced the miRNA levels of LPL and the STAT3-regulated genes STAT3, Bcl2, c-Myc, cyclin D1, p21/WAF1, and VEGF. Furthermore, unlike the empty vector, STAT3 shRNA downregulated STAT3 and LPL protein levels by 80% (Fig. 5C). Taken together, our findings suggest that STAT3 binds and activates the LPL promoter and induces the expression and production of LPL.

**Discussion**

In this study, we found that CLL cells, unlike normal B lymphocytes but similar to adipocytes and myocytes, store lipids in cytoplasmic vacuoles and are capable of metabolizing stored FFAs in an LPL-dependent manner.

Traditionally, CLL has been characterized by an accumulation of immunologically abnormal, long-lived lymphocytes (29). However, data generated in the past decade showed that approximately 1% of CLL cells are regenerated daily (5). Various cells use different strategies to provide energy to fuel proliferation (30). One such strategy, used physiologically by adipocytes and myocytes, is the generation of cytoplasmic lipid stores that serve as a readily available energy source. This strategy has been adopted by various cancer cells. For example, the “starry sky” pattern, characterized found in Burkitt’s lymphoma, is caused by an abundance of intracellular lipid vacuoles used as an energy source in this highly aggressive lymphoma (31). We found that CLL cells, similar to Burkitt’s lymphoma cells but unlike normal B lymphocytes, store lipids in a form of cytoplasmic lipid vacuoles. We could not study lymph node CLL cells. However, because CLL cells traffic between the lymph nodes blood and bone marrow (1), it is reasonable to assume that lymph node CLL cells carry similar features. Remarkably, it was found that the gene expression profile of CLL cells is skewed toward the expression of genes usually expressed in muscle and fat tissue (32). It is therefore likely that to adjust for cellular survival and a proliferation rate higher than in their cell of origin, CLL cells adopted the strategy of mammalian muscle cells and adipocytes, which store intracellular lipids and proliferate at similar rates (33).

Similar to adipocytes, CLL cells express LPL on their surface and in their cytoplasm and store lipids in cytoplasmic vacuoles. However, the functional significance of the expression of LPL in CLL cells is not entirely clear. Overexpression of lipase activity–associated genes (34) and increased lipase activity were found in CLL cells (34). Conversely, other investigators suggested that LPL is catalytically inactive in CLL cells (35) and that LPL knockdown did not affect the survival of CLL cells (36). Here, we show that LPL provides CLL cells with survival advantage. Transfection of CLL cells with LPL-siRNA increased CLL cell apoptosis rate by an average of 32%. Whether this effect is induced by an abrogation of the catalytic or noncatalytic function of LPL remains to be determined.

FFAs, the product of triglyceride hydrolysis by LPL, are the fuel used for oxidative phosphorylation, and are also required to prompt the enzymatic machinery needed for their own metabolism. FFAs bind the PPAR-activated alpha (PPARα) which translocates to the nucleus and induces the transcription enzymes that are needed for fatty acid oxidation (37). Remarkably, PPARα was found to be expressed by CLL cells, and CLL cell palmitic acid oxidation rate was similar to the oxidation rate typically found in fat-burning cells (38).

Our previous studies suggested that the unique gene signature of CLL is driven in part by constitutively activated STAT3 (19, 20, 39, 40). In the current study, we show that STAT3 binds to the LPL promoter and activates the transcription of LPL. Transfection of CLL cells with STAT3-shRNA significantly downregulated LPL mRNA and protein levels, suggesting that the transcription of LPL in CLL cells is STAT3 dependent. STAT3 is not the only transcription factor that induces LPL gene expression. Abreu and colleagues (41) suggested that in CLL patients with unmethylated IgH microenvironment-induced demethylation contributes to the increased expression of LPL in CLL. Nonetheless, our data suggest that the survival advantage provided by constitutively activated STAT3 (14) is mediated in part by the contribution of STAT3 to the CLL cells’ metabolism.

Taken together, our findings suggest that CLL cells store lipid vacuoles, produce LPL, and adapt their metabolism to utilize intracellular stored lipids for energy production in an LPL-dependent manner, a process that is driven by STAT3.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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