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Metabolism pathways in chronic lymphocytic leukemia

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Abstract

Alterations in CLL cell metabolism have been studied by several investigators. Unlike normal B lymphocytes or other leukemia cells, CLL cells, like adipocytes, store lipids and utilize free fatty acids (FFA) to produce chemical energy. None of the recently identified mutations in CLL directly affects metabolic pathways, suggesting that genetic alterations do not directly contribute to CLL cells' metabolic reprogramming. Conversely, recent data suggest that activation of STAT3 or downregulation of microRNA-125 levels plays a crucial role in the utilization of FFA to meet CLL cells' metabolic needs. STAT3, known to be constitutively activated in CLL, increases the levels of lipoprotein lipase that mediates lipoprotein uptake and shifts CLL cells' metabolism towards utilization of FFA. Herein we review the evidence for altered lipid metabolism, increased mitochondrial activity, and formation of reactive oxygen species in CLL cells, and discuss possible therapeutic strategies to inhibit lipid metabolism pathways in patient with CLL.

Keywords

Metabolism; CLL; STAT3; Lipoprotein lipase; Oxidative phosphorylation

Introduction

Various tumorigenic alterations significantly affect neoplastic cell metabolism. Although generally heterogeneous, tumorigenic modifications often converge at similar metabolic pathways [1]. Whether these metabolic deviations, typically identified in rapidly proliferating cells, alter the metabolism of low grade, slow growing tumors is yet unknown.

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Traditionally, CLL has been perceived as a disease in which slowly proliferating neoplastic lymphocytes accumulate and do not die because of an inherent defect in their apoptotic machinery [2–4]. Evidence from recent years suggest that CLL cells undergo spontaneous apoptosis [5] and that the gradual increase in the size of the CLL clone results from newly born lymphocytes [6]. Unlike resting B-lymphocytes, approximately 0.1 % to 1.75 % of CLL cells proliferate daily [6], and the cells' metabolic program is reprogramed to adjust for their increased proliferation rate. Similar to myocytes and adipocytes, which proliferate at similar rates, CLL cells store lipids in cytoplasmic lipid vacuoles and the CLL cell gene expression profile is skewed towards increased expression of genes that are usually expressed in muscle and fat tissues [7].

This review focuses on the metabolic pathways utilized by CLL cells and the adaptation CLL cells make to meet their increased metabolic demands [8].

Carbohydrate metabolism

Glycolysis is the metabolic pathway that converts glucose into pyruvate. Whereas under aerobic conditions the pyruvate dehydrogenase complex oxidizes pyruvate in the inner membrane of the mitochondria to form acetyl CoA, under anaerobic conditions lactate dehydrogenase catalyzes the conversion of pyruvate to lactic acid.

In 1921 Braunstein found that glucose was no longer detected in the urine of diabetic patients after they developed cancer. To test whether cancer cells consume sugar, he cultured normal and malignant tissues in a glucose rich solution and found that cancerous tissue consumed more sugar than normal tissue [9,10]. In 1923 Otto Warburg reported that in tumor tissue glucose is converted to lactate even in the presence of oxygen and named this phenomenon aerobic glycolysis [11]. Aerobic glycolysis was detected in acute leukemias and in a variety of lymphoproliferative neoplasms [12]. Additionally, the total glycolytic activity was found to be a good predictor of outcome in patients with diffuse large B cell lymphoma [13].

Studies in the 60th and the early 70th documented an impaired glucose tolerance test in patients with CLL [14] and demonstrated that glucose uptake is reduced in CLL cells compared to normal B lymphocytes [15,16]. Contrary to these findings, recent studies suggested that the glycolytic pathway is operative in CLL cells from most patients and that CLL cells have the capacity to utilize glucose and produce lactate [17,18]. Similarly, a tonic activation of the B cell receptor was found to activate the glycolysis pathways in lymphoma cell lines [19].

Conversely, studies evaluating glucose metabolism in-vivo by using positron emission tomography and uptake of 2-Deoxy-2-[^{18}F] fluoroglucose (FDG) in CLL found a low degree of ^{18}F-FDG avidity and low sensitivity of PET-FDG [20–22]. Unlike in CLL, high avidity was detected in other lymphoproliferative diseases. Thus, while some degree of activity of the glycolytic pathway is evident in CLL, this pathway does not seem to play a key role in CLL cells' metabolism, unlike its role in other rapidly proliferating lymphoproliferative neoplasms. In fact, when the neoplastic cells exhibit high FDG uptake,

it is strongly suggestive for Richter's transformation or the development of an aggressive lymphoma, typically diffuse large B cell lymphoma [23].

Oxidative phosphorylation in CLL

The chemiosmotic theory

The central role of adenosine triphosphate (ATP) in cellular metabolism was discovered in 1941 [24]. Twenty years later Peter D. Mitchel proposed the chemiosmotic coupling theory offering a link between oxidation and phosphorylation [25]. Highly controversial at first, this theory gradually became universally accepted. According to the chemiosmotic theory the synthesis of ATP in respiring cells originates from nicotineamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2), formed from breakdown of energy-rich molecules. The name chemiosmotic is derived from the association between two processes, the flow of hydrogen ions according to an electrochemical concentration gradient and osmosis and the diffusion of water across selective permeable membranes. This flow provides ample energy to phosphorylate ADP to produce ATP and generate water by pairing protons with oxygen [25]. In aerobic organisms chemiosmosis across the inner membrane of the mitochondria drives oxidative phosphorylation, the metabolic pathway in which oxidation of various 'nutrients' is used to generate Not surprisingly, mitochondria play a pivotal role in providing cancer cells ATP. The proton flow drives ATP synthase, a large enzymatic complex which serves as a rotary mechanical motor and phosphorylates ADP. Whereas 2 ATP molecules are formed by anaerobic fermentation during glycolysis, oxidative phosphorylation is significantly more efficient because 30 to 36 ATP molecules are formed from a single glucose molecule. One hundred thirty eight ATP and 9 GTP molecules are formed in beta-oxidation of C18:0 (stearic acid) while oxidation of three molecules of glucose (total 18 carbons) produce 108 ATP and 6 GTP molecules. Therefore oxidation of fatty acids yields more ATP than oxidation of glucose.

Mitochondrial activity and the formation of reactive oxygen species (ROS)

Human mitochondrial DNA (mtDNA) encodes 37 genes, 13 of which transcribe oxidative phosphorylation enzymes [26]. Early reports suggested that leukemia cells harbor alternate mtDNA structures such as circular dimers, catenated dimmers and trimmers [27]. mtDNA is more amenable to mutations than nuclearDNA (nDNA) because it lacks the chromatin organization of nDNA [28]. Although chemotherapy was shown to induce heterotrophic mtDNA mutations in CLL [29], Meierhofer et al. found that blood transfusions might have 'contaminated' the tested samples with donors' blood cell mitochondria [30] harboring abnormalities in hot spots that are frequently mutated in the general population [31]. Nevertheless, although the mtDNA structure of patients with CLL is no different from that of normal individuals, several studies showed that an increase in mtDNA copy numbers is associated with an increased risk for the development of CLL and non-Hodgkin's Lymphoma [32,33].

That mitochondrial function might be relevant to carcinogenesis was first proposed by Otto Warburg [34]. To synthesize ATP through oxidative phosphorylation, the mitochondria consume most of the cellular oxygen and, as byproducts, produce reactive oxygen species

(ROS) [35]. The carcinogenic potential of ROS was attributed to its capacity to damage macromolecules and nucleic acids [36], thereby inducing an accumulation of mutations and other genetic abnormalities [36]. High ROS levels were found to be associated with defective cell-signaling [37], genetic instability [38], and cancer cell immune escape resulting from natural killer (NK)- and/or T-cell dysfunction [39–42]. Contrariwise, increased ROS levels are accompanied by increased antioxidant activity therefore high ROS levels might inhibit tumorigenesis [43].

Recent studies suggest that CLL cells adjust to their increased energy demands by increasing their mitochondrial activity. For example, Jitschin et al. found that the number of mitochondria, the total mitochondrial mass, mitochondrial biogenesis, mitochondrial electron transport activity, and mitochondrial membrane potential are increased in CLL cells compared to normal B lymphocytes [44]. These findings indicate that mitochondrial respiration rate is increased in CLL cells and, as a result, the levels of mitochondria-derived ROS are higher in CLL cells than in normal B cells [44–46]. Moreover, when CLL cells are deprived of glucose their mitochondrial oxidative phosphorylation is further increased [47].

ROS and the microenvironment

Activation of the BCR and related pathways is thought to protect CLL cells from apoptosis [48]. Conversely, ROS display a dual effect in CLL cells. ROS promote tumor progression by modifying the microenvironment and concurrently facilitate apoptotic cell death. Elevated ROS levels likely contribute to the lymph node's protective milieu [49,50]. Therefore strategies to exploit the aberrant redox characteristics of CLL cells in order to induce apoptosis have been proposed [51]. Similarly, stromal cells provide cysteine for the synthesis of the antioxidant glutathione and, as a result, relieving ROS-derived oxidative stress [52].

Because immune cells are sensitive to ROS-induced damage [53], high levels of ROS contribute to the impaired immune surveillance observed in patients with CLL [54,55]. For example, CLL patients with high ROS levels have reduced numbers of activated CD4+ cells and exposure to an antioxidant restores T-cell function [44]. ROS-induced immune deregulation is probably mediated by modified DNA and lipids detected in CLL patients' plasma [46]. However, the accumulation of ROS induces apoptosis and antioxidants may protect CLL cells from apoptosis. For example, N-acetylcysteine known to block the generation of ROS, protected CLL cells from apoptotic cell death [56].

Altered Lipid Metabolism in CLL

The carcinogenic potential of most oncogenes has been attributed to their ability to sustain proliferation, regulate cell cycle and evade cell death [57,58]. However data accumulated in recent years suggest that the oncogenic potential of several proteins whose levels are increased in neoplastic cells, is associated with reprogramed cellular metabolism [58]. A gene expression analysis revealed that CLL cells' signature is similar to that of fat or muscle cells [7]. Particularly, lipoprotein lipase (LPL), normally expressed in adipocytes and muscle cells[59], was found to be aberrantly expressed in CLL cells [60–62]. Increased LPL mRNA levels correlated with an aggressive disease and unfavorable prognosis [4,60–63]

whereas in CLL patients with a mutated IgHV, low LPL mRNA levels a hypermethylated *LPL* promoter was found [64,65].

To determine why LPL is aberrantly expressed in CLL we analyzed the ENCODE project's chromatin immunoprecipitation followed by high-throughput DNA sequencing (ChIP-seq) data [66]. We found that none of the transcription factors (TF) that bind the LPL promoter are known to be operative in CLL. Therefore we analyzed the 1.5 kilobase length sequence upstream the LPL gene and, using the PROMO software, identified several putative TF-binding sites [67,68]. Two of those were putative binding sites of the nuclear factor of activated T-cells (NFAT) and two were putative binding sites of signal transducer and activator of transcription (STAT)-3, both of which are known to be operative in CLL.

Since their discovery more than 20 years ago, members of the STATs family were found to play a crucial role in the pathogenesis of most malignancies [69]. In CLL, STAT3 is constitutively phosphorylated on serine 727 residue [70,71] and activates genes that provide CLL cells with survival advantage [71–74]. Indeed, we have recently found that in CLL phosphoserine STAT3 binds to and activates the promoter of lipoprotein lipase (LPL) [75].

LPL has non-catalytic and catalytic functions. It induces cellular uptake of lipoproteins and prompts the hydrolysis of triglycerides into free fatty acids (FFAs) [76]. Driven by constitutively activated STAT3, LPL induces storage of lipoproteins in cytoplasmic lipid vacuoles and reprograms CLL cells to preferentially use lipids as an energy source. Although levels of LPL are higher in IgHV-unmutated cases, utilization of FFA is operative in CLL regardless of clinical characteristics or IgHV mutation status.

In newly diagnosed CLL patients the levels of cholesterol, high density lipoprotein-cholesterol (HDL-C), very low density lipoprotein-cholesterol (VLDL-C) and triglycerides are relatively low [77], likely because of an increased uptake of cholesterol mediated by LPL. Lipid-mediated signaling might be disrupted in CLL cells. For example the expression of sphingosine 1-phosphate receptor-1, known to mediate lipid-dependent signaling, is low in CLL patients' lymph nodes and upon BCR inhibitor treatment its level increases, likely contributing to mobilization of CLL cells from lymph nodes to the peripheral blood [78].

Unlike normal B lymphocytes CLL cells store lipids in cytoplasmic vacuoles and utilize FFAs to produce energy via oxidative phosphorylation [75]. Metabolomic analysis of CLL cells identified increased levels of FFAs and triglyceride degradation products, suggesting that these changes are induced by downregulation of microRNA (miR)-125 and a reciprocal increase in lipolysis-facilitating enzymes [79]. FFAs, the substrate for oxidative phosphorylation, are also ligands of the nuclear receptor peroxisome proliferator activated receptor (PPAR)-α. After FFAs bind PPARα, the FFA-PPARα complex functions as a transcription factor and activates the transcription of enzymes necessary for oxidative phosphorylation [80]. Hence, LPL generates FFAs through its catalytic activity thereby supplying fuel for oxidative phosphorylation, and in addition, drives the transcription of PPARα (Figure 1). Remarkably, PPARα is overexpressed in circulating CLL cells and its levels correlate with advanced-stage disease [81].

Thus, STAT3-driven aberrantly expressed LPL, plays a major role in metabolic reprogramming by skewing the metabolism of CLL cell towards utilizing lipids. This phenomenon provides a rational for targeting lipid metabolism in CLL cells.

Targeting metabolic pathways in CLL cells

Statins are competitive inhibitors of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase. This enzyme plays a key role in the production of cholesterol in the liver. Statins have also been shown to induce the production of the LDL receptor which draws cholesterol out of the circulation. While reducing blood cholesterol levels statins also inhibit prenylation of proteins known to be operative in in signal transduction pathways [82].

Statins were found to inhibit proliferation of cancer cells and these in vitro results were supported by clinical data. For example, Nielsen et al. reported that statin administration reduced cancer-related mortality [83]. Whether statins directly target metabolic pathways is still unknown. However, muscle cells of patients with statin-induced myopathy express low levels of oxidative phosphorylation-related genes [84].

In CLL cells statins induces apoptosis [85,86] likely by inducing the activation of the mitochondrial caspase-9 [86]. Although statins did not affect the clinical outcome of newly diagnosed patients with early stage CLL [87], retrospective data analysis suggested that the administration of statins and aspirin was associated with improved outcome in patients with relapsed/refractory CLL treated with salvage chemotherapy [88].

The anti-diabetic drug metformin was found to inhibit the mitochondrial respiratory chain and consequent mitochondrial oxidative phosphorylation [89]. Additionally, its use was associated with a reduced incidence of cancer in patients with diabetes [90]. Because metformin inhibits oxidative phosphorylation in CLL cells and possesses a potential anti-leukemic activity [91], clinical trials of metformin in CLL have been initiated https://umclinicalstudies.org/HUM00061356, https://clinicaltrials.gov/ct2/show/NCT01750567.

Other approaches to target oxidative phosphorylation in CLL by blocking fatty acid metabolism using the PPARα antagonist MK886 [92] or using PK11195, a benzodiazepine derivate that blocks the mitochondrial F1F0-ATPase [44], have been reported.

Although carbohydrate metabolism appears to play a minor role in CLL cell metabolism, the human immunodeficiency (HIV) protease inhibitor ritonavir decreased CLL cell viability possibly by inhibiting the activity of Glucose transporter type 4 (GLUT4), hence targeting glucose transport into CLL cells [91]. In another approach nanoliposomes containing the sphingolipid metabolite Ceramide were used to induced necrosis of CLL cells by inhibiting glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a glycolytic pathway enzyme found to be overexpressed in CLL [18] (Table 1).

Conclusions

Warburg's original hypothesis that a genetic alteration in a metabolic pathway results in the development of cancer, has recently gained renewed traction. For example, the discovery of

activating mutations in isocitrate dehydrogenases (IDH1) and in methylcytosine dioxygenase (TET2) in AML and MDS [93,94] supports Warburg's hypothesis and led to the development of targeted therapies that are currently investigated in clinical trials. Conversely, none of the recurrent mutations detected in CLL cells is directly involved in altering metabolic pathways [95,96], suggesting that CLL cells' metabolic reprogramming is not directly induced by genetic aberrations. Although epigenetics reprogramming of CLL cell metabolism is an appealing theory, there are no data to support this hypothesis and, thus far, STAT3 and miR-125 are the two known master regulators of cellular metabolism identified in CLL cells. STAT3 transduces signals through the JAK/STAT signaling pathway and acts as a transcription factor [97]. STAT3, constitutively activated in CLL cells, activates the LPL gene that shift the metabolic program of CLL cells towards the utilization of lipids. MiR-125 contributes to the metabolic adaptation of B lymphocytes and CLL cells and its downregulation leads to a transformed state [79].

Metabolism, broadly defined as the sum of biochemical processes that either produce or consume energy [98], is essential for the survival of every living organism. Alterations in the function of master regulators of metabolism might protect cells from apoptosis-inducing insults or initiate cellular transformation. An in-depth understanding of these processes will likely provide us with potential targets for therapeutic interventions in CLL.

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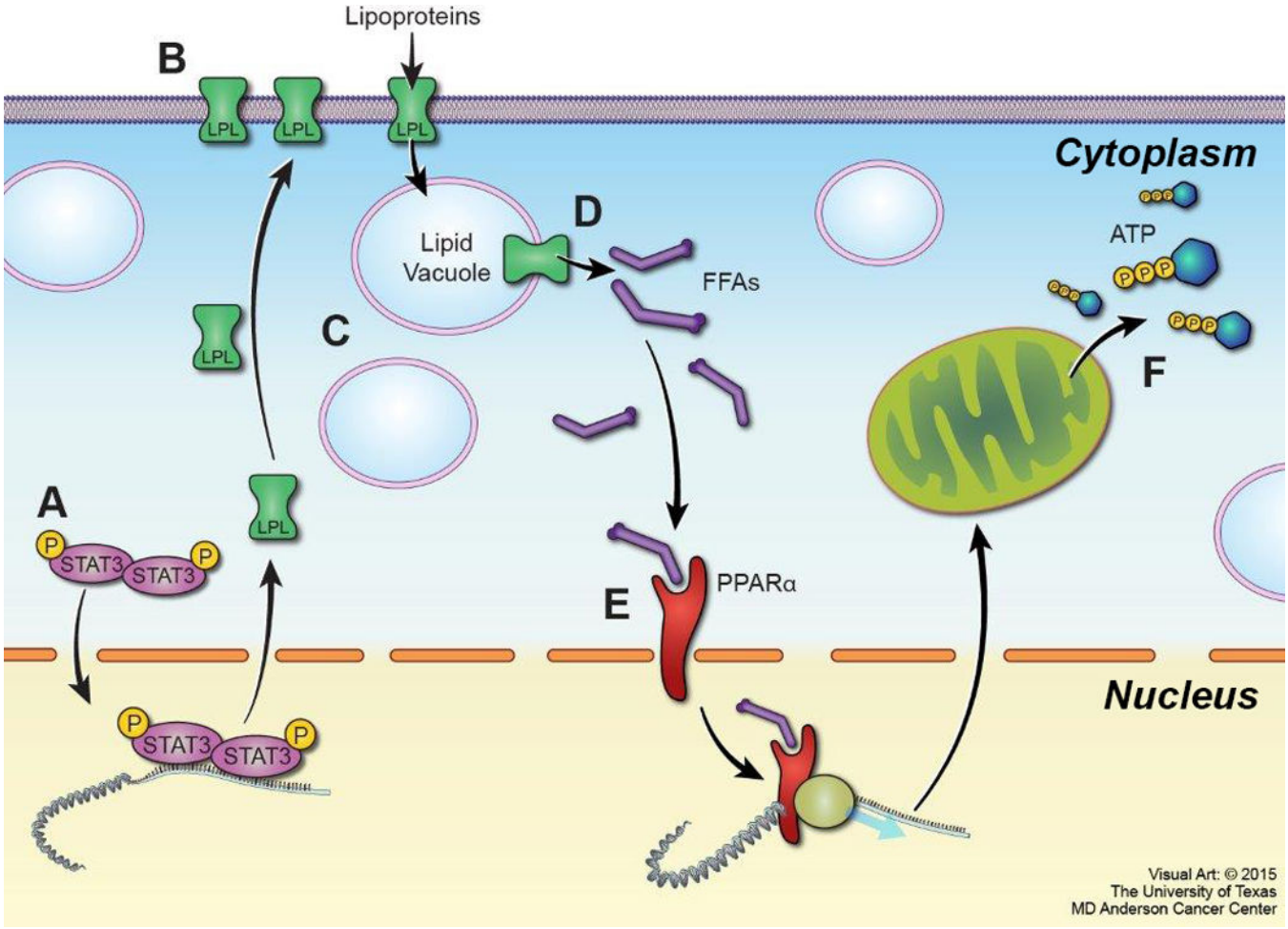

Figure 1. Model of lipid metabolism in CLL cells

A. In CLL cells signal transducer and activator of transcription (STAT)-3 is constitutively phosphorylated on serine 727 residues. Phosphorylated STAT3 forms dimers, shuttles to the nucleus, binds to DNA and activates STAT3-target genes. **B**. Because lipoprotein lipase (LPL) is a STAT3-target gene, LPL is aberrantly expressed in CLL cells. LPL is found in the cytosol and cell membrane of CLL cells and mediates cellular uptake of lipoproteins. **C**. Unlike in normal B lymphocytes, in CLL cells lipid vacuoles are scattered in the cytoplasm. **D**. LPL hydrolyzes triglycerides stored in lipid vacuoles into free fatty acids (FFAs). **E**. FFAs bind to and prompt proliferator-activated receptor (PPAR)-α shuttle into the nucleus. PPARα activates genes oxidative phosphorylation genes. **F**. PPARα-target gene proteins enter the mitochondria and induce oxidative phosphorylation.

Table 1

Drugs and Chemical Compounds that Target Metabolic Pathways and Were Tested in CLL

Compound name	Class	Primary Mechanism of Action	Possible Effect in CLL	Status in CLL	References
Simvastatin	Statins	Competitive inhibitors of HMG-CoA reductase	Reduces viability and increases apoptosis rates of CLL cells	Retrospective analysis of patients' data	[86], [85],[88]
Atorvastatin				Drug tested in-vitro	
Metformin	Biguanide	Anti-diabetic Suppressing gluconeogenesis	Inhibition of mitochondrial OP	Drug tested in-vitro	[47]
Rosiglitazone	Thiazolidinedione	Anti-diabetic, Decreases insulin resistance	Decreases expression of CD5 and ZAP70, Induces apoptosis	Drug tested in-vitro	[85]
MK886	Leukotriene inhibitor	Inhibition of PPARα activity	Kills resting CLL cells and causes immunogenic death of proliferating CLL cells	Compound tested in-vitro	[92]
PK11195	Benzodiazipine derivate	Generates mitochondrial superoxide by inhibiting the mitochondrial F1F0-ATPase	Blocks OP and induces cell death	Compound tested in-vitro	[44]
Ritonavir	Protease inhibitor	Inhibits cytochrome P45—3A4 (CYP3A4)	Inhibits the glucose transporter GLUT4, inhibits OP and potentiates metformin-induced apoptosis	Drugs tested In-vitro	[47]
Ceramide	Waxy lipid	Found at high concentrations in the cell membrane and participate in cellular signaling	Inhibits GAPDH, induces necrosis of CLL cells	Compound tested in-vitro	[18]

OP, oxidative phosphorylation ; PPARα, peroxisome proliferator-activated receptor-α; GAPDH, glyceraldehyde 3-phosphate dehydrogenase