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Metabolic rewiring beyond Warburg in chronic lymphocytic leukemia: How much do we actually know?

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ABSTRACT

Chronic Lymphocytic Leukemia (CLL) is the most common adult leukemia in the western world. CLL consists of the accumulation of malignant B-cells in the blood stream and homing tissues. Although treatable, this disease is not curable, and resistance or relapse is often present. In many cancers, the study of metabolic reprograming has uncovered novel targets that are already being exploited in the clinic. However, CLL metabolism is still poorly understood. The ability of CLL lymphocytes to adapt to diverse microenvironments is accompanied by modifications in cell metabolism, revealing the challenge of targeting the CLL lymphocytes present in all different compartments. Despite this, the study of CLL metabolism led to an ongoing clinical trial using glucose uptake and mitochondrial respiration inhibitors. In contrast, glutamine and fatty acid metabolism remain to be further exploited in CLL. Here, we summarize the present knowledge of CLL metabolism, as well as the metabolic influence of Myc, ATM and p53 on CLL lymphocytes.

1. Introduction

Chronic Lymphocytic leukemia (CLL) is characterized by the clonal expansion and accumulation of malignant B-Cell lymphocytes in the blood stream and in homing tissues (such as bone marrow and lymphoid organs). In circulation, CLL lymphocytes are quiescent and dependent on intrinsic survival factors. In contrast, these cells can proliferate when they enter homing tissues, revealing a challenge for the design of therapeutic interventions that target both intrinsic and microenvironmentally promoted survival pathways (Messmer et al., 2005).

Metabolic rewiring is an important hallmark of cancer (Hanahan and Weinberg, 2011), which allows the cells to modify their metabolism and fulfill the requirements needed to sustain survival, quiescence, differentiation or proliferation. The changes in metabolic phenotypes can contribute to both, transformation and tumor progression (DeBerardinis and Chandel, 2016; Cluntun et al., 2017). Although this knowledge is already being applied for prognostic, diagnostic and therapeutic purposes (Koglin et al., 2011), it is important to consider that each type of cancer has distinct features and modifies its metabolism in response to diverse stimuli (such as nutrient availability, oncogenic activation, proliferative state or microenvironment) (Wolpaw and Dang, 2017; Gentric et al., 2017).

The understanding of quiescent cell metabolism – relevant in CLL – is still incomplete (Rozovski et al., 2016), limiting the potential therapeutic use of metabolic inhibitors for CLL. CLL lymphocytes display heightened mitochondrial respiration, elevated levels of reactive oxygen species (ROS), and enhanced antioxidant capacity (e.g. glutathione) (Jitschin et al., 2014; Mayer et al., 2018; Tili et al., 2012; Carew et al., 2004), compared to normal B-cells. In spite of having an operative glycolytic pathway (Jitschin et al., 2014; Tili et al., 2012), CLL lymphocytes do not follow the Warburg effect (with no enhanced lactate production) (Martinez Marignac et al., 2013). Currently, metabolic based approaches are being tested in the clinic for CLL. These procedures are based on preclinical results (Jitschin et al., 2014; Martinez Marignac et al., 2013; Adekola et al., 2015) – ours included – targeting mitochondrial respiration and glucose uptake utilizing the partial mitochondrial Complex I inhibitor: metformin and the glucose uptake inhibitor: ritonavir (www.clinical.trials.gov) (Galicia-Vazquez et al., 2018).

This review recapitulates various studies in CLL metabolism, with a focus on central carbon metabolism, as well as the influence of deletions spanning the ATM (del11q) and TP53 (del17p) tumor suppressors, associated with fast disease progression and poor response to standard-of-care treatment (Oscier et al., 2002; Gladstone et al., 2012). Metabolite flux analysis studies are very limited in CLL; thus, we will discuss available studies utilizing metabolic modulators, targeted

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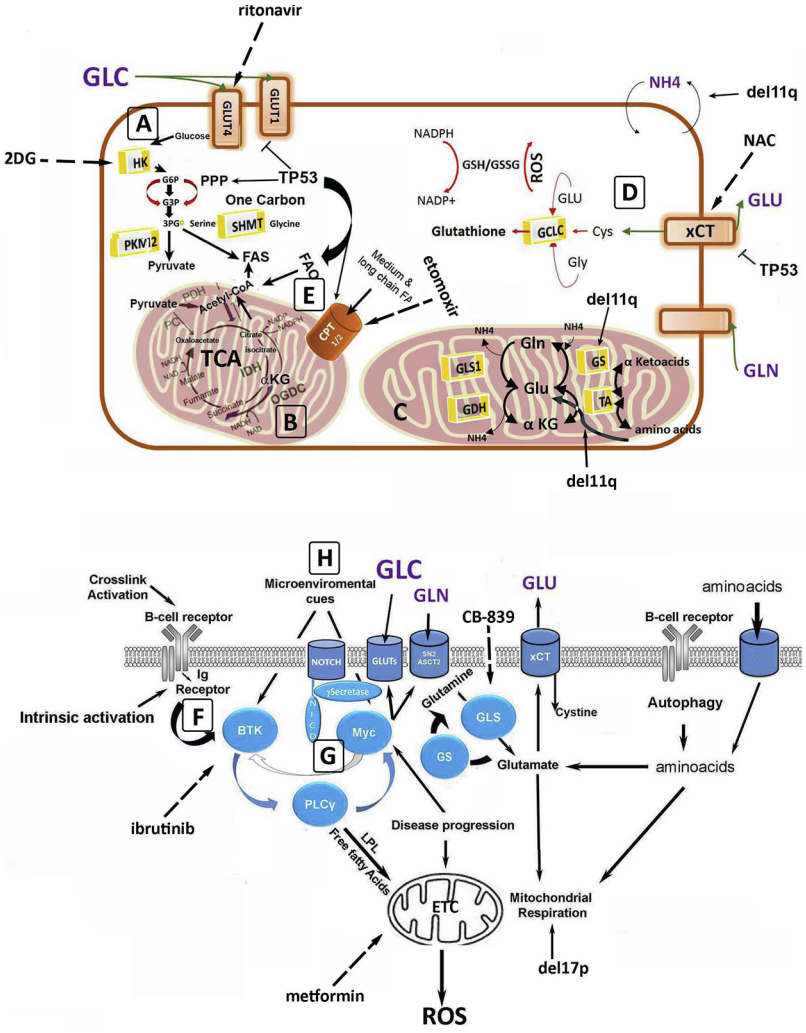

Fig. 1. Networks affecting metabolism with a focus on central carbon metabolism in CLL.
The processes discussed in the text are identified with the same letter as follows:**Upper Panel: A** Glucose metabolism, **B** Mitochondrial tricarboxylic acid (TCA) cycle, **C** Mitochondrial amino acid metabolism, **D** xCT dependent-glutathione synthesis and **E** Fatty acid synthesis (FAS) and mitochondrial fatty acid oxidation (FAO).
Lower Panel: F Bruton's tyrosine kinase (BTK) is activated downstream of the B cell receptor (BCR), in both ligand dependent and independent manner (intrinsic). *In vitro* ligand-dependent BCR activation is mimicked by crosslinking of the receptor and it is believed to be mediated by autoantigens *in vivo*. BCR engagement enhances Myc downstream signaling in a BTK dependent manner in the presence or absence of stromal cell support. **G** Conversely, whether BTK is activated downstream Myc in CLL- as reported in premalignant B cell is- not known.
H Microenvironmental clues- mimicked by stromal cell support *in vitro*-enhance both BTK and Myc signaling. Although the role of Myc in metabolic rewiring to stromal cell support has been described in CLL, the role of BTK in these process has not been yet explored.
Metabolic modulators and abbreviations:
Ritonavir is an inhibitor of GLUT4, 2DG is a glucose analogue that inhibits glycolysis, CB-839 is an inhibitor of GLS1, Etomoxir is a CPT1 inhibitor, NAC is a cystine precursor, ibrutinib is an irreversible BTK inhibitor affecting glucose, amino acids and fatty acid metabolism. GLC (Glucose), GLU (Glutamate), GLN (Glutamine), ETC (electric transport chain).

metabolomics and exometabolomic tools.

2. Glucose metabolism in CLL (Figure 1A)

Glucose-derived carbons can be catabolized via glycolysis and the pentose phosphate pathway. As well, glucose can be utilized for glycogen, fatty acid and serine synthesis (e.g. one carbon cycle). Each pathway favors distinct processes and metabolite intermediates.

Although the role of glycogen synthesis in CLL lymphocytes has been suggested by seminal studies (Mitus et al., 1958), it has not been revisited systematically since the seventies). Glucose metabolism, and especially the dependence of some malignancies on glucose availability has increased interest in this area. Since glucose dependency is not a generalized feature in tumor cells, alternative ways to target glucose metabolism could be relevant for therapeutic purposes .

2.1. Glycolysis

The notion that cancer cells can reprogram their metabolism has been present for a long time. One of the first observations of metabolic rewiring was the Warburg effect, which involves an increase in glycolytic flux, reflected in the secretion of pyruvate-derived lactate to the extracellular media (aerobic glycolysis) (Ward and Thompson, 2012). The increase in glycolytic flux allows the cell to produce ATP and metabolites to fulfill high proliferation demands, rendering these cells addicted to glucose. CLL cells take up glucose and have operational glycolysis; however, they do not seem to follow the Warburg effect. In line with reported limited aerobic glycolysis (Rozovski et al., 2016), it has been documented that lactate secretion is within the nanomolar range (per 10^6 cells), while glucose uptake falls into the micromolar range in primary CLL lymphocytes (Galicia-Vazquez et al., 2018; Marignac et al., 2013). Although quiescent, glucose metabolism is elevated in CLL lymphocytes (Tili et al., 2012; Koczula et al., 2016) with a further increase when cultured with stromal cells (Jitschin et al., 2015). In line with this, inhibitors of glucose uptake (ritonavir) (Adekola et al., 2015; Galicia-Vazquez et al., 2018) and glycolysis (2DG) were reported to induce *in vitro* cytotoxicity in CLL (Martinez Marignac et al., 2013; Galicia-Vazquez et al., 2018).

Apart from their known role in DNA damage response and cell cycle control, *ATM* and *TP53* are regulators of central carbon metabolism and redox homeostasis (Zaugg et al., 2011; Berkers et al., 2013; Krüger and Ralser, 2011; Vousden and Ryan, 2009). Therefore, their deletion in CLL could lead to targetable metabolic processes. Deletion of *ATM* causes an increase in insulin receptor expression and consequently glucose uptake (Saiya-Cork et al., 2011), possibly leading to an increase in glucose dependency in de11q CLL lymphocytes. In line with this, CLL primary lymphocytes positive for del11q are particularly sensitive to glycolysis inhibition (Galicia-Vazquez et al., 2018).

TP53 favors catabolic pathways, and its deficiency or deletion confer more dependency on glucose metabolism (Matoba et al., 2006; Ogasawara et al., 2016). Metabolic effects by TP53 (reviewed elsewhere (Napoli and Flores, 2017)) include modulation of glucose uptake and catabolism. TP53 limits glucose uptake via repression of GLUT1 and GLUT4 and glycolysis by activating the transcription of TIGAR. TIGAR,

(TP53-inducible regulator of glycolysis and apoptosis) is expressed by primary CLL lymphocytes (Martinez Marignac et al., 2013) and has been shown to promote the use of glucose-6-phosphate through the pentose phosphate pathway (Napoli and Flores, 2017) (see below).

2.2. Pentose phosphate pathway (PPP)

Glucose can be used as a source of metabolic intermediates for glycolysis and for the pentose phosphate pathway as well. This pathway redirects glucose-derived carbons to produce reducing equivalents and nucleotides for nucleic acid synthesis. The first reaction of this pathway is catalyzed by G6PDH, which can be inhibited pharmacologically *in vitro*. Curtailing the pentose phosphate pathway (Lemons et al., 2010) is not cytotoxic to CLL cells (Galicia-Vazquez et al., 2018); however, it does increase basal metabolic activity, suggesting the redirection of glucose carbons to TCA cycle, leading to an increase in NADH production (Galicia-Vazquez et al., 2018). Thus, it is possible that glycolysis and PPP carbons shuttle from one pathway to another upon metabolic stress. In line with this idea, the combination of glycolysis and PPP inhibitors is highly cytotoxic (and synergistic) to CLL lymphocytes.

2.3. One carbon metabolism

Recently, one carbon metabolism has brought attention as an important target for cancer (Locasale, 2013). The targeting of one carbon metabolism has been present for more than 50 years, by interfering with folate synthesis using methotrexate. Methotrexate is an analogue of folic acid, employed in the treatment of a variety of cancers, including childhood ALL (Kager et al., 2005).

The impact of one carbon cycle on CLL metabolism remains to be extensively explored. *In vitro*, CLL lymphocyte viability is not inhibited by AMPA, an inhibitor of SHMT1/2 (enzyme that catalyzes the conversion between serine and glycine (Li et al., 2013)), but it antagonizes the cytotoxicity induced by glycolysis inhibition (Galicia-Vazquez et al., 2018). The latter suggests that glycolysis and serine synthesis compete for glucose-derived carbons through PKM2 activity modulation (Chaneton et al., 2012; Cheng et al., 2011), and that AMPA redirects these carbons back to glycolysis. Further investigation will be needed to confirm this idea.

3. Mitochondrial metabolism in CLL (Figure 1B)

Mitochondria play a very important role in cell metabolism, they are involved in a variety of processes such as oxidative phosphorylation, oxidative stress control, plus metabolite and energy production – via the TCA cycle. As well, they are implicated in amino acid and fatty acid metabolism.

CLL cells have an increased mitochondrial number, mass, activity and ROS production compared to normal B-cells (Jitschin et al., 2014). In line with this, the pharmacological inhibition of oxidative phosphorylation (PK11195, oligomycin A and metformin) is cytotoxic to CLL lymphocytes (Jitschin et al., 2014; Martinez Marignac et al., 2013; Adekola et al., 2015). Regarding TCA cycle metabolism, CLL lymphocytes overexpress oxoglutarate dehydrogenase (OGDC) and isocitrate dehydrogenase (IDH), two enzymes that can facilitate the TCA cycle flux in the forward and reverse directions (oxidation or reduction of α-ketoglutarate) (Zelenetz, 2017). Although this observation supports the idea of metabolic plasticity in CLL, flux analysis studies, using isotopically labeled TCA cycle substrates, are needed to clarify this possibility.

It has been demonstrated that TP53 contributes to the maintenance of mitochondrial integrity (Napoli and Flores, 2017). In proliferating human cancer cells, TP53 deficiency can be compensated by an increase in aerobic glycolysis (Ogasawara et al., 2016). In contrast, CLL lymphocytes compensate for TP53 deficiency by an enhancement of mitochondrial mass. In a murine model, null TP53 CLL lymphocytes have reduced expression of components of the mitochondrial electric transport chain (ETC) enhanced basal oxygen consumption rate (OCR) and ATP content, plus increased mitochondrial mass. Consistent with this, del17p positive primary CLL lymphocytes express heightened proliferator-activated receptor gamma coactivator 1-α (PGC-1α), a major regulator of mitochondrial biogenesis (Napoli and Flores, 2017).

3.1. Amino acid metabolism (Figure 1C)

Metabolite levels and availability have an impact on intracellular signaling cascades and redox state amongst other cellular processes. Apart from glucose, cancer cells can use amino acids (i.e. glutamine) to obtain the necessary energy and building blocks to survive and proliferate. Amino acids are derived from different intracellular or extracellular sources. In CLL patients, decreased levels of isoleucine and increased levels of pyruvate and glutamate (Glu) in the plasma of affected patients have been documented (MacIntyre et al., 2010). Although not experimentally confirmed, this agrees with transamination of alanine to α-ketoglutarate, resulting in the production of glutamate and pyruvate. Depending on metabolic rewiring, α-ketoglutarate (produced from the catabolism of amino acids) can contribute to the replenishment of the TCA cycle or can be extracted for fatty acid or glutamate synthesis (Owen et al., 2002). Using NanoString technology, it was shown that CLL lymphocytes display heightened expression of mitochondrial IDH3 and citrate transporter (SLC25A1) which yield α-ketoglutarate from isocitrate and cytoplasmic export of citrate respectively (Zelenetz, 2017).

3.1.1. Glutamine/glutamate metabolism

Glutamine is the most abundant amino acid in the blood, present at ~0.5-1 mM. Despite being a non-essential amino acid, it has been observed that K-Ras and Myc-driven tumors can become addicted to glutamine for survival (Gaglio et al., 2011; Wise et al., 2008). The first and limiting step of glutamine catabolism is the conversion of glutamine to glutamate by glutaminase (GLS). Glutaminase mRNA is overexpressed in CLL and has been targeted by various inhibitors *in vitro*. Moreover, the glutaminase inhibitor CB-839 is in clinical trials for AML and ALL treatment (clinicaltrials.gov) (Bromley-Dulfano et al., 2013).

Apart from glutaminase, CLL lymphocytes overexpress glutamate dehydrogenase, the enzyme catalyzing the conversion of glutamate to α-ketoglutarate; the glutamate-cystine antiporter; and glutamate-cysteine ligase, the rate-limiting enzyme of glutathione synthesis (Mayer et al., 2018). In CLL lymphocytes, enhanced Myc signaling, a promoter of glutamine metabolism in other cancer cells, is associated with disease progression and with metabolic rewiring by stromal cells (Jitschin et al., 2015; Gao et al., 2009). However, a direct link between Myc signaling and the observed increase in glutamine metabolism needs to be confirmed experimentally.

<u>*Del11q*</u> CLL lymphocytes exhibit differential glutamine metabolism compared to their negative counterparts. Del11q CLL lymphocytes have increased ammonia uptake and glutamine synthetase expression, favoring *de novo* glutamine synthesis. It has been recently reported that CLL lymphocytes positive for del11q are particularly sensitive to a glutaminase inhibitor, paving the way for the targeting of glutamine metabolism in this subset (Galicia-Vazquez et al., 2018).

3.1.2. Cystine-glutamate antiporter (Figure 1D)

Previous reports show that CLL lymphocytes accumulate extracellular glutamate *in vitro* (Koczula et al., 2016; MacIntyre et al., 2010; Piszcz et al., 2013), which is consistent with the overexpression of the cysteine-glutamate (xCT) antiporter by CLL cells *in vivo* and *in vitro* (Galicia-Vazquez et al., 2018; Koczula et al., 2016; MacIntyre et al., 2010). Apart from being a substrate for TCA cycle, glutamate contributes to redox homeostasis by feeding glutathione synthesis. In line with this, ROS levels are raised by both glutaminase inhibition and by glutamine depletion but not by interference with glucose metabolism.

Additionally, glutamate deamination – via glutamate dehydrogenase – can contribute to glutathione regeneration by providing NADPH (co-factor in the reduction of oxidized glutathione) (Altman et al., 2016).

CLL lymphocytes, as shown in other cancer cell types, produce glutamate to import cystine via the xCT antiporter (Tili et al., 2012). The xCT antiporter ensures cystine import necessary for mitochondrial glutathione synthesis and ROS control. Hence, extracellular cystine can reduce intracellular glutamate levels by stimulating xCT activity (Muir et al., 2017). In other cancer types, the stimulation of xCT system reduces metabolic plasticity, enhancing glutamine dependency (Muir et al., 2017; Koppula et al., 2018). On the other hand, xCT inhibition is cytotoxic for some proliferating cancer cells, involving enhancement of peroxidized lipids (abundant in CLL) and glutathione depletion (Coscia et al., 2012).

Interestingly, while increased ROS levels can be compensated by a cysteine precursor (NAC), the cytotoxicity derived from glutaminase inhibition is exacerbated by NAC. NAC increases glutamine uptake and glutamate secretion, suggesting a compensatory mechanism to regenerate glutamate pools. This process is likely curtailed by glutaminase activity inhibition, leading to cell death (Galicia-Vazquez et al., 2018). Currently, pharmacological stimulators and inhibitors of xCT antiporter are commercially available, opening a window of opportunity for the design of new therapeutic approaches targeting cystine-glutamate antiport system. Interestingly, their effect on CLL lymphocyte metabolism and survival has not been evaluated yet. Because TP53 represses xCT expression, xCT overexpression and hypersensitivity to xCT inhibition have been reported in other cancer cell types expressing mutated TP53 (Oscier et al., 2002; Koppula et al., 2018; Pekarsky and Croce, 2015; Ouillette et al., 2011). Thus, xCT is a target of interest in CLL, especially to target del17p cases.

4. Fatty acid metabolism in CLL (Figure 1E)

The contribution of fatty acid metabolism to CLL metabolic plasticity has been previously explored. Apart from the dependency of CLL lymphocytes on fatty acids for membrane remodeling (Spaner et al., 2013), active fatty acid synthesis (FAS) and fatty acid oxidation (FAO) is proposed by metabolomic studies in primary CLL lymphocytes and by the cytotoxicity of inhibitors of FAS or FAO (Rozovski et al., 2016; Spaner et al., 2013; Pallasch et al., 2008; Tung et al., 2013). Fatty acid synthesis is favored via citrate production by isocitrate dehydrogenase, both overexpressed in CLL (Mayer et al., 2018; Tili et al., 2012). Fatty acid synthesis can be inhibited using Orlistat, reportedly cytotoxic to primary CLL lymphocytes *in vitro* (Pallasch et al., 2008). In contrast, the over-expression of enzymes associated with enhanced fatty acid oxidation in CLL has also been reported (Mayer et al., 2018; Tili et al., 2012). CPT-1 is highly expressed in primary CLL lymphocytes and mediates the mitochondrial import of fatty acids for FAO (Marignac et al., 2013). Etomoxir targets fatty acid oxidation via CPT-1 inhibition and is cytotoxic to primary CLL lymphocytes. The latter is accompanied by an elevation in ROS levels (Galicia-Vazquez et al., 2018), a possible consequence of an imbalance between fatty acid synthesis and oxidation (Galicia-Vázquez and Aloyz, 2018). In addition to the PPP, fatty acid synthesis and oxidation can contribute to stabilize ROS levels by consuming NADPH, or by indirectly increasing NADPH levels respectively (Carracedo et al., 2013).

5. Autophagy

Autophagy can be an alternative source of metabolites, such as amino acids or fatty acids. It has been reported that dasatinib treatment induces autophagy in CLL lymphocytes resistant to the tyrosine kinase inhibitor. Furthermore, inhibition of autophagy sensitized primary CLL lymphocytes to dasatinib. Similarly, it is possible that TP53 deficiency by del17p results in reduced metabolic plasticity (i.e. impaired autophagy) increasing the susceptibility of del17p CLL samples to limited nutrient availability (Amrein et al., 2011; Amrein et al., 2009).

6. B-Cell receptor (BCR)-dependent metabolic rewiring in CLL (Figure 1F)

BCR signaling is of relevance for CLL treatment because it promotes survival, migration, and proliferation of malignant B-cells (Woyach and Johnson, 2015). In CLL, both ligand independent and antigen-induced BCR activation are present, supported by constitutive phosphorylation of BCR signaling components (Dühren-von Minden et al., 2012; Gobessi et al., 2009). The Bruton's tyrosine kinase (BTK) is a component of BCR signaling (Hendriks et al., 2014), activated by tonic BCR signaling (intrinsic activation) and by micro-environmental cues in homing tissues (which are believed to include autoantigens) (Burger, 2016). BTK is a relevant target in CLL as it can be pharmacologically inhibited by ibrutinib, an irreversible BTK inhibitor that causes lymphocytosis and accelerated cell death of circulating CLL cells (Ponader et al., 2012). Although not curative, ibrutinib is a leading compound in the treatment of CLL and other hematological malignancies. Generally, acquired resistance to ibrutinib occurs due to mutations in BTK or its target: PLCγ (Woyach et al., 2014; Lampson and Brown, 2018). The mechanisms contributing to *DeNovo* resistance to ibrutinib are ill defined, however inferior clinical responses to first line ibrutinib treatment have been linked to chromosomal abnormalities (Amin et al., 2015; Jain et al., 2015).

In addition to curtailing BCR signaling, ibrutinib promotes metabolic responses, revealing the participation of BTK in metabolic control. Ibrutinib increases glucose and glutamine uptake as well as ROS levels of *in vitro* treated CLL lymphocytes (Galicia-Vazquez et al., 2018; Vaisitti et al., 2017). Furthermore, ibrutinib-induced cytotoxicity is enhanced when combined with inhibitors of glucose uptake but antagonized by a ROS scavenger (Galicia-Vazquez et al., 2018). Recent publications suggest that ibrutinib affects fatty acid metabolism because the drug induced inhibition of free fatty acid synthesis (Rozovski et al., 2018) and enhanced dependency on fatty acid oxidation (Galicia-Vázquez and Aloyz, 2018). However, the mechanism by which ibrutinib affects fatty acid metabolism is still not known. Additionally, the influence of ibrutinib on CLL lymphocyte metabolic fluxes remains to be elucidated. Ibrutinib was shown to reduce Notch signaling in CLL in the presence or absence of stromal cells (see below), supporting that cross talk between BTK and Notch/Myc signaling exists in CLL (Secchiero et al., 2017).

7. Role of Myc in CLL metabolism (Figure 1G)

In other cancer cells, Myc can increase glutamine uptake and glutaminase expression, and has been linked to ibrutinib resistance via constitutive activation of BTK and PLCϒ2 (Moyo et al., 2017). Whether Myc plays such a role in CLL remains to be explored. In non-transformed B cells, however, Myc seems to contribute to ibrutinib resistance via enhancement of BTK and PLCγ2 activation (Moyo et al., 2017). In proliferating cancer cells, Myc raises glutamine and/or glucose metabolism, mitochondrial biogenesis and drives glutamine addiction. Myc promotes mitochondrial glutamine utilization by increasing the expression of glutamine transporters (e.g. SN2 and ASCT2) and glutaminase (GLS1) (Wise and Thompson, 2010; Le et al., 2012). Glutamine addiction has been associated with limited TCA cycle anaplerosis – rescued by supplementation with α-ketoacids – and with limited capacity to achieve *de novo* glutamine synthesis. However, the role of Myc in quiescent cancer cell metabolism is not fully characterized (Miller et al., 2012; Wang et al., 2008). Myc expression in CLL rises with disease progression, independent of other prognostic factors (e.g del11q, del17p or IgVH status) (Zhang et al., 2010). In CLL, BCR engagement enhances Myc expression in a BTK dependent manner as it is abrogated by ibrutinib (Yeomans et al., 2016).

Richter syndrome is the development of an aggressive lymphoma in

patients with chronic lymphocytic leukemia (CLL). Richter transformation, occurring in 5–10% of CLL cases, has an unfavourable prognosis. Similar to CLL, metabolic alterations have been detected in Richter syndrome (Bruzzi et al., 2006), such as enhanced glucose metabolism. Also, mutations in Myc have been linked to Richter syndrome (Rossi and Gaidano, 2018).

8. Role of the microenvironment in CLL metabolism. (Figure 1H)

In CLL the stromal-cell microenvironment promotes survival through the activation of PI3K and BTK, and by promoting redox homeostasis. Bone marrow stromal cells import cystine and convert it to cysteine, which is then released into the microenvironment for uptake by CLL cells to promote glutathione synthesis (Zhang et al., 2012). Myc contributes also to metabolic adaptations to stromal-cell microenvironment in CLL. Stromal cells (6 days co-culture) promote an increase in the glycolytic capacity without affecting oxidative phosphorylation in CLL lymphocytes. This glycolytic switch depends on Notch-c-Myc signaling regardless of IgVH status. Stromal cells enhance glucose uptake, GLUT3 expression, ATP content, and increases the expression of glycolytic enzymes (such as hexokinase-2, lactate dehydrogenase A, pyruvate dehydrogenase kinase-1, enolase-1, and glyceraldehyde-3-phosphate dehydrogenase) without reducing oxidative phosphorylation (Jitschin et al., 2015). Therefore, it was proposed that the targeting of glucose metabolism or the Notch-c-Myc signaling pathway could be exploited to interfere with stromal cell–mediated CLL drug resistance. It was recently shown that pharmacologic interference with Notch signaling by a γ-secretase enhances ibrutinib cytotoxicity in CLL cells co-cultured with stromal cells, supporting the notion of a cross talk between BTK and Notch/Myc signaling in CLL (Secchiero et al., 2017). In contrast, a different report showed that short term co-culture (e.g. 24–48 h) augmented ATP levels by stromal cell interaction, associated with enhanced oxidative phosphorylation plus a heterogeneous but non-significant effect on glucose and glutamine uptake or ROS levels (Vangapandu et al., 2017).

9. Therapeutic targeting of metabolic pathways

Current clinical trials targeting CLL lymphocyte bioenergetics involve the use of the OXPHOS inhibitor metformin (NCT01750567) alone or in combination with the GLUT4 inhibitor ritonavir (NCT02948283). Based on the metabolic traits discussed above, it can be anticipated that del17p cases will be more sensitive to metformin, alone or in combination with ritonavir. Also, this subset might show hypersensitivity to xCT inhibitors. On the other hand, glutaminase and asparaginase inhibitors could target del11q cases. Asparagine is a non-essential amino acid present in circulation. It has been reported that some tumors, especially leukemias, cannot synthesize asparagine. Therefore, asparagine depletion from blood – using L-asparaginase – has been implemented for ALL treatment (Pui and Evans, 2006). L-asparaginase is also able to catalyze the conversion of glutamine to glutamate, which could translate into a therapeutic option for glutamine-addicted tumors. Since del11q CLL lymphocytes are over-sensitive to glutaminase inhibition, asparaginase treatment could represent a treatment option for targeting these cells.

Regarding ibrutinib, its combination with ritonavir or an xCT inhibitor could be clinically tested, to potentiate the effects of the kinase inhibitor. Furthermore, curtailing fatty acid oxidation could be tested to enhance ibrutinib cytotoxicity, and to sensitize *de Novo* ibrutinib resistant CLL lymphocytes. Because, available reports indicate that metabolic rewiring in CLL is heterogeneous, systematic metabolomic and proteomic studies are needed to define subsets by their metabolic liabilities.

10. Concluding remarks

Since CLL circulating lymphocytes are considered quiescent, the analysis of CLL metabolic rewiring needs to be addressed in a different manner than highly proliferating tumors. Interestingly, it has been noticed that CLL cells exposed to high (12.5 mM) or limited (2 mM) glucose levels display different metabolic responses (Martinez Marignac et al., 2013). In accordance to this idea, alternative metabolic rewiring was documented in cancer cell lines exposed to physiological concentrations of metabolites in the media (Cantor et al., 2017). The use of physiological metabolite concentrations in culture media could be of relevance for obtaining an accurate profile of metabolic rewiring of CLL lymphocytes. Through CLL research, the claims that either fatty acid or glutamine metabolism (or even glucose uptake) are the key targets to combat CLL are often contradictory. Despite the advances in CLL metabolism, a wide metabolomics analysis that closely correlates to patient outcome could greatly contribute to clarify CLL lymphocyte metabolic rewiring and help define future therapeutic targets to fight the disease effectively.

Conflict of interest

The authors do not have conflict of interest to declare.

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