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ENERGY METABOLISM CHANGES IN B- AND T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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ABSTRACT

Tomi Viitanen: Energy Metabolism Changes in B- and T-cell Acute Lymphoblast Leukemia
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Leukemias are cancers occurring in the hematopoietic system of especially children and adolescents. The most common leukemia is acute lymphoblastic leukemia (ALL), which covers approximately 80% of leukemia cases in children and adolescents. Even though the treatment and prognosis of it has improved notably over the past decades, still one in ten cases relapses or leads to death. With older patients the malignancy is rarer, but the treatment is more challenging.

Metabolic alterations are one of the hallmarks of cancer. The study of these is one of the oldest areas of cancer research, with the aim of identifying metabolic features, signals, and mutations that are specific to cancer cells. This information is used to understand how metabolic changes give cancer cells an advantage in surviving difficult conditions as well as proliferating uncontrollably. The best-known discovery in this area is the Warburg effect, which is a highly conserved characteristic in almost all cancers, including ALL. Warburg effect describes the phenomenon in which cancer cells produce large amounts of lactate even in oxygen-rich conditions. Normally, this only happens when there is not enough oxygen available to maintain oxidative phosphorylation.

Changes in the metabolism of cancer cells have been observed during disease progression, chemotherapy, and in disease recurrence. These metabolic changes affect the phenotype of cancer cells and can also influence treatment response, potentially even causing drug resistance. Therefore, understanding specific metabolic changes in ALL cells is important for developing targeted therapeutic approaches. Recent studies have identified several key changes in ALL cell's metabolic pathways, which provide essential advantages for cell survival and development. Since these pathways are often critical for cell survival, they can also be targeted and utilized in cancer treatment. Many specific drugs targeting cell metabolism are being developed, some of which have shown promising results in preclinical studies of ALL. Simultaneously targeting different pathways has also been found to be a promising strategy in research. Targeting certain metabolic pathways has been found to resensitize cells to chemotherapy.

Targeting cell metabolism is a promising strategy in cancer treatment. It can enhance therapeutic effects by being used alongside traditional chemotherapy or be integrated into it to treat certain subtypes of ALL. This can improve the effectiveness of ALL treatment and additionally prevent or reverse drug resistance, the leading cause of mortality in leukemia.

The aim of this literature review is to investigate changes in energy metabolism in B- and T-ALL cells and provide a comprehensive overview of them. In addition, metabolic features that could serve as targets for drug treatments will be identified, various drugs targeting different metabolic pathways will be searched for, and their effects on ALL cells will be examined.

Keywords: ALL, leukemia, energy metabolism, metabolic reprogramming, targeted drugs

TIIVISTELMÄ

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Leukemiat ovat erityisesti lapsilla ja nuorilla ilmentyviä hematopoeettisessa systeemissä esiintyviä syöpiä. Leukemioista yleisin on akuutti lymfoblastileukemia (ALL), joka kattaa lapsilla ja nuorilla noin 80 % leukemiatapauksista. Vaikka kyseisen sairauden hoito ja ennuste on viime vuosikymmenien aikana parantunut huomattavasti, vieläkin noin yksi kymmenestä tapauksesta uusiutuu, tai johtaa kuolemaan. Iäkkäämmillä potilailla tauti on harvinaisempi, mutta usein vaikeahoitoisempi.

Aineenvaihdunnan muutokset ovat yksi syövän tunnusmerkeistä. Niiden tutkiminen on yksi vanhimmista syövän tutkimusaloista, jonka tavoitteena on tunnistaa syöpäsoluille ominaisia aineenvaihdunnan piirteitä, signaaleja ja mutaatioita, jotka aiheuttavat aineenvaihdunnan muutoksia. Tätä tietoa käytetään selvittämään, miten aineenvaihdunnan muutokset luovat etulyöntiaseman soluille selviytyä vaikeissa olosuhteissa, sekä lisääntyä hallitsemattomasti. Tunnetuin löydös on Warburgin efekti, joka on hyvin konservoitunut piirre lähes kaikissa syövissä, myös ALL:ssa. Warburgin efekti kuvaa ilmiötä, jossa syöpäsolut tuottavat suuria määriä laktaattia hapellisissa olosuhteissa. Laktaatin tuotantoa tapahtuu normaalisti vain, kun happea ei ole tarjolla riittävästi oksidatiivisen fosforylaation ylläpitämistä varten.

Syöpäsolujen aineenvaihdunnan muutoksia on huomattu tapahtuvan taudin kehityksessä, kemoterapian aikana, sekä taudin uusiutuessa. Nämä aineenvaihdunnan muutokset vaikuttavat syöpäsolujen fenotyyppiin ja voivat myös vaikuttaa hoidon vasteeseen, pahimmillaan aiheuttaen lääkeresistenssiä. Siksi ALL-soluissa esiintyvien spesifisten aineenvaihdunnan muutosten ymmärtäminen on tärkeää kohdistettujen terapeuttisten lähestymistapojen kehittämisessä. Viimeaikaiset tutkimukset ovat tunnistaneet useita keskeisiä muutoksia ALL-solujen aineenvaihduntareiteillä. Nämä muutokset tuovat olennaisia etuja solujen selviytymiselle ja kehittymiselle. Sen vuoksi ne voivat toimia syövän heikkouksina lääkehoidoissa. Spesifisiä solun aineenvaihduntaan kohdistuvia lääkkeitä on kehitteillä monia, joista osa on tuottanut lupaavia tuloksia ALL:n prekliinisissä malleissa. Eri reittien samanaikainen kohdentaminen on myös todettu lupaavaksi strategiaksi tutkimuksissa. Tiettyihin aineenvaihduntareitteihin kohdistuvan lääkinnän on huomattu uudelleen herkistävän solut kemoterapialle.

Solun ainevaihduntaan kohdistuva lääkintä on lupaava strategia syövän hoidossa. Se voi toimia perinteisen kemoterapian ohella tai siihen integroituna hoitamaan tiettyjä ALL:n alatyyppejä voimistaen terapeuttista vaikutusta. Sen avulla pystytään nostamaan ALL-hoidon tehoa entiseltään ja ehkäistä tai peruuttaa lääkeaineresistenssiä – suurinta kuolleisuuden aiheuttajaa leukemiassa.

Tämän kirjallisuuskatsauksen tavoitteena on tutkia B- ja T-ALL-solujen energia-aineenvaihdunnan muutoksia ja maalata kattava yleiskuva niistä. Lisäksi tavoitteena on tunnistaa aineenvaihdunnan piirteitä, jotka voisivat toimia lääkehoitojen kohteina, etsiä eri aineenvaihduntareitteihin kohdistuvia lääkeaineita ja tarkastella niiden vaikutuksista ALL-soluihin.

Avainsanat: ALL, leukemia, energia-aineenvaihdunta, aineenvaihdunnan muutokset, kohdennettu lääkehoito

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Tomi Viitanen

CONTENTS

1. INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant disorder of the hematopoietic system and the most common cancer amongst children and adolescents (Malard and Mohty, 2020, Soltani *et al.* 2021). In ALL rapidly and aggressively proliferating malignant and immunologically inactive lymphoblasts are produced (Soltani *et al.* 2021). ALL can be categorized into two main subtypes, B-ALL, and T-ALL, depending on whether the malignancy originates from immature B- or T-lineage cells. ALL arises from early lymphoid cells in the bone marrow whose differentiation becomes blocked at an early stage (Malard and Mohty, 2020).

While tremendous progress has been made in treating and curing this disease over the years, with a 5-year overall survival rate reaching 90% in children, relapse and therapy resistance remain major obstacles in treatment (Malard and Mohty, 2020, Soltani *et al.* 2021). One area of research, which could further advance understanding of ALL progression and provide novel treatments, is the metabolic reprogramming of ALL cells. Alterations in the energy metabolism, contribute to ALL cell survival, growth, and therapy resistance (Sbirkov *et al.* 2020). By providing essential energetic advantages to malignant cells, the metabolic pathways could be a prominent target in the treatment of ALL.

Metabolic reprogramming is a hallmark of cancer and aids cells to adapt to increased energy needs and changing microenvironments allowing uncontrolled proliferation (Kumar *et al.* 2022). One of the oldest areas in cancer research is cancer metabolism. The field has been largely based on the findings from Otto Warburg who observed that tumor metabolism shift into producing high quantities of lactate even in aerobic conditions, more commonly known as the Warburg effect or aerobic glycolysis (Warburg *et al.* 1927). Glycolysis and lactate production is a natural response in hypoxic conditions, but what Warburg observed was happening regardless of oxygen availability (DeBerardinis and Chandel, 2016). This feature is a prominent feature in ALL along with many other alterations (Kishton *et al.* 2016, Rashkovan and Ferrando, 2019, Sbirkov *et al.* 2020).

This review aims to investigate the metabolic reprogramming that occur in B- and T-ALL and how they contribute to disease progression as well as treatment response. By examining the underlying metabolic pathways and main signaling networks involved in ALL pathogenesis, this review seeks to provide a better understanding of the disease and its relation to metabolic features, to identify potential metabolism-related targeted therapies and recognize items that are unknown or poorly studied in the field.

2. METABOLISM IN HEALTHY AND CANCER CELLS

Metabolism is the process by which cells convert nutrients into energy and building blocks essential for growth, maintenance, and energy. The metabolic pathways involved in this process are complex and tightly regulated, ensuring that cells have the energy and materials they need to function properly. In cancer, the regulation of these pathways can become disrupted, leading to abnormal metabolism that supports the growth and survival of cancer cells (DeBerardinis and Chandel, 2016, Kumar *et al.* 2022) (figure 1). Likewise, healthy immune cells like lymphocytes also have unique metabolic requirements to carry out their specialized functions in the body. In this chapter, we will explore the intricacies of energy metabolism in healthy and cancerous cells, as well as the metabolic adaptations of lymphocytes in response to various stimuli.

Glycolysis is the process by which glucose is broken down into pyruvate, producing two ATP molecules and two NADH molecules (Pearce *et al.* 2013). This process is essential to produce energy for cells. Pyruvate can be further metabolized through two main pathways: 1) mitochondrial citric acid cycle (TCA) and oxidative phosphorylation (OXPHOS) pathway 2) or lactate production (Pearce *et al.* 2013). In addition to producing many intermediate metabolites for anabolic metabolism, TCA cycle produces additional ATP molecules by oxidizing pyruvate, and OXPHOS maximizes the energy produced from glucose totaling in 34-36 ATP molecules produced from one molecule of glucose (Pearce *et al.* 2013). Lactate production occurs when oxygen supply is limited and contributes to energy production.

The metabolic pathways commonly affected in cancer cells are glycolysis, pentose phosphate pathway (PPP), TCA, glutaminolysis, OXPHOS, and fatty acid oxidation (FAO) (DeBerardinis and Chandel, 2016). Reprogramming counteracts factors such as physiological stress and the need for metabolic intermediates essential for cell growth and proliferation (Pearce *et al.* 2013, Rashkovan and Ferrando, 2019, Soltani *et al.* 2021). In addition to rapidly generating ATP, aerobic glycolysis produces intermediates that can be utilized in the anabolic pathways of cells (Pearce *et al.* 2013, Rashkovan and Ferrando, 2019). This is believed to be one of the reasons why aerobic glycolysis is a common metabolic characteristic in all types of cancers, despite not being the most efficient bioenergetic pathway (Rashkovan and Ferrando, 2019). Moreover, PPP generates intermediates for nucleotide biosynthesis while generating NADPH (Pearce *et al.* 2013). NADPH has a dual effect; it is essential in nucleotide and fatty acid synthesis (FAS) in addition to guarding cells against oxidative stress (Pearce *et al.* 2013, Rashkovan and Ferrando, 2019).

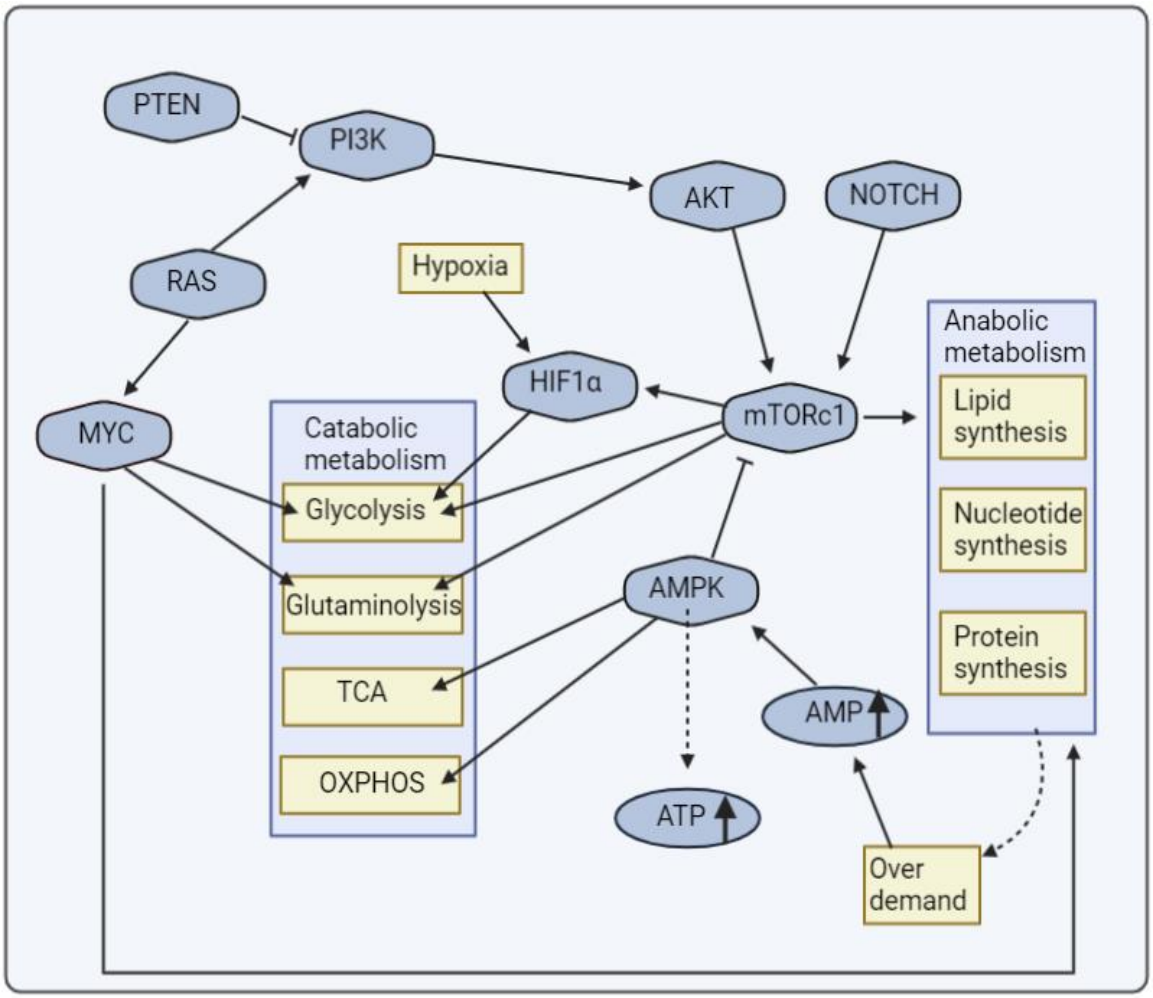

Figure 1. *A simplified picture pathways affecting cancer cells metabolic reprogramming. mTORC1 activates anabolic metabolism and glycolysis. However, over demand of anabolic metabolism leads to energy stress and the consequent AMPK activation which inhibits it while increasing mitochondrial activity. adenosine monophosphate (AMP), AMP dependent kinase (AMPK), protein kinase B (Akt, PKB), adenosine triphosphate (ATP), hypoxia-inducible factor 1-alpha (HIF1α), mammalian target of rapamycin complex 1 (mTORC1), oxidative phosphorylation (OXPHOS), phosphoinositol triphosphate kinase (PI3K), phosphatase and tensin homolog (PTEN), reticular activating system (RAS). Image created with BioRender.com.*

Metabolic reprogramming is one of the hallmarks of cancer. In an ALL-sequencing study consisting of 2000 children 23 different subtypes of ALL were identified (Gu *et al.* 2019). One connecting characteristic between each of these subtypes was deregulation of metabolism (Gu *et al.* 2019, Sbirkov *et al.* 2020). Deregulated metabolism allows the uncontrolled proliferation of cancer cells and accumulation of tumor biomass. Without metabolic reprogramming the cells cannot satisfy the increased energy needs required for a malignant transformation (DeBerardinis and Chandel, 2016, Chan *et al.* 2017). Some reprogrammed pathways provide essential advantages for the cancer cell and as such can be effective targets for inhibitory therapies (Rashkovan and Ferrando, 2019). Other altered pathways might be initially disposable but become prevalent when the microenvironment changes – upon metastasis or metabolic stress (DeBerardinis and Chandel, 2016, Kishton *et*

al. 2016). The fundamental understanding of different metabolic pathways intermediates, regulators, and functions is important for the development of new therapies.

Different proteins and signaling pathways take part in cancer cell metabolism reprogramming, such as PTEN, HIF1α, PI3K-AKT, and NOTCH (DeBerardinis and Chandel, 2016, Kishton *et al.* 2016, Sbirkov *et al.* 2020, Grüninger *et al.* 2022). These proteins affect one another leading to the regulation of cellular metabolism (Figure 1). Inactivation or hyperactivation in these is essential for metabolic reprogramming. Similar inactivations or hyperactivations are found between types of cancer – such as PI3K-Akt pathway hyperactivation is found in 88% of B-ALL and NOTCH pathway activating mutations found in 60% of T-ALL cases (Kishton *et al.* 2016, Grüninger *et al.* 2022). Thus, emerging types of metabolism profiles emerge, some of which can be exploited in the treatment of cancer.

The metabolism of hematopoietic stem cells (HSCs) is characterized by anaerobic glycolysis, which results in relatively low levels of mitochondria derived reactive oxygen species and ATP production (Soltani *et al.* 2021). Reactive oxygen species are produced in mitochondrial metabolism indicating a decreased level of OXPHOS. Low oxygen microenvironment in the bone marrow results in hypoxia-inducible factor 1-alfa (HIF1α) stabilization and the consequent glycolytic phenotype promotion and TCA inhibition (Soltani *et al.* 2021). Leukemic stem cells (LSCs) share many similarities with HSCs in terms of their metabolic profile. However, LSCs are characterized by even greater reliance on anaerobic glycolysis and lower levels of mitochondrial energy production compared to HSCs (Soltani *et al.* 2021).

Recent studies have shown metabolic reprogramming and lymphocyte activation are closely connected (Pearce *et al.* 2013, Kishton *et al.* 2016). Quiescent T-cells maintain low rates of glucose uptake and mainly utilize it through OXPHOS, meanwhile also leveraging FAO (Pearce *et al.* 2013). Resting T-cells respond to antigen-specific signals initiating drastic reprogramming of cells metabolism to support rapid proliferation and differentiation (Pearce *et al.* 2013). Like many cancerous cells, activated proliferating T-cells metabolism shifts to a highly increased aerobic glycolysis (Pearce *et al.* 2013, DeBerardinis and Chandel, 2016, Kishton *et al.* 2016, Kumar *et al.* 2022).

In hematologic malignancies, aerobic glycolysis is not only energetically and anabolically favorable, but it also provides advantages trough the high level of lactate and hydrogen ion secretion (Soltani *et al.* 2021). Secretion of hydrogen ions decreases pH of the surrounding environment providing advantages to leukemia cells (Soltani *et al.* 2021). Acidic, high lactate microenvironment is detrimental to immune cells targeting leukemia cells (Soltani *et al.* 2021). High concentrations of lactate have shown suppressing effects on monocyte differentiation, dendritic cell and effector T-cell cytokine secretion (Soltani *et al.* 2021). Furthermore, it impairs T-cell function by hindering their concentration gradient dependent lactate transportation leading in accumulation of intracellular lactate

(Soltani *et al.* 2021). NK cells are also affected by this microenvironment. High acidity reduces NK cells cytotoxicity (Soltani *et al.* 2021). As Treg cells energy production is driven by FAO, this microenvironment does not affect their function as much, leading to a constant expression of Treg immunosuppressive functions. (Soltani *et al.* 2021) Overall, this microenvironment derived from high rates of aerobic glycolysis in ALL cells impairs immune function creating favorable conditions for leukemia cell invasion.

3. METABOLIC REPROGRAMMING IN ALL

3.1 Metabolism in B-ALL cells

In 2006, Boag et al. were the first to prove an aberrant metabolism in B-ALL cells. Gene expression analysis of 22 cALL samples showed upregulation in genes promoting glycolysis in B-ALL specimens compared to healthy CD34+ cells (Boag *et al.* 2006). B-ALL cells showed increased aerobic glycolysis while being less dependent on energy production from TCA cycle (figure 2). Inhibition of glycolysis with 2-deoxy-D-glucose (2DG) or Lonidamine largely decreased ALL cell viability and induced apoptosis while TCA inhibition with oligomycin alone did not affect growth of B-ALL cell line (Boag *et al.* 2006, Buentke *et al.* 2011, Martín-Lorenzo *et al.* 2018, Rashkovan and Ferrando, 2019, Mirabilii *et al.* 2020). Although TCA is not the main source of energy, it is maintained by other intermediates such as carbon derived from glutaminolysis (Sbirkov *et al.* 2020, Soltani *et al.* 2021).

Aerobic glycolysis is the driving force of energy production and source of nutrient intermediates for anabolic metabolism in B-ALL (Mirabilii *et al.* 2020, Soltani *et al.* 2021). Upregulation in Glut1 and -4 transporters promotes glycolysis by enabling increased uptake of glucose (Boag *et al.* 2006). In addition, the greater level of N-terminal glycosylation of Glut1 transporters increases binding affinity to glucose (Boag *et al.* 2006). Upregulation in enzymes of glycolysis (PFKL and hexokinase (HK)) were observed while enzymes of TCA cycle (such as IDH3B, FH, and MDH1/-2) were downregulated (Boag *et al.* 2006, Sbirkov *et al.* 2020) (figure 2). Three of four subunits of the PDH complex, which functions in catalyzing pyruvate into acetyl-CoA, were downregulated which is an essential step for products of glycolysis to enter the TCA (Boag *et al.* 2006).

Shift to aerobic glycolysis is essential for B-ALL cells to make a malignant transformation (Chan *et al.* 2017). This can be mediated trough mutations in many different genes. Most notable of these are disrupting mutations in *PAX5, IKZF1, EBF1 and TCF3* (Chan *et al.* 2017, Martín-Lorenzo *et al.* 2018) or hyperactivity of PI3K-Akt pathway and RAS (Sbirkov *et al.* 2020, Grüninger *et al.* 2022). Hyperactivity of the PI3K-AKT pathway correlates with a poorer prognosis and is reported in 88%

of B-ALL cases (Sanchez *et al.* 2019, Grüninger *et al*. 2022). Under hypoxic conditions signaling trough PI3K-Akt pathway with MAPK activation stabilizes HIF1α and induces glycolytic phenotype (Mirabilii *et al.* 2020). PI3K-Akt pathway activation has been linked to B-cell survival, differentiation, and altered metabolism (Sanchez *et al.* 2019). PTEN, a main negative regulator of PI3K-Akt pathway, interestingly is not often mutated in B-ALL (Sanchez *et al.* 2019). Interplay between HIF1α, hypoxic environment and mTOR leads to upregulation of glycolytic enzymes including HK, PFKFB3, S6K1 and PKM (Mirabilii *et al.* 2020).

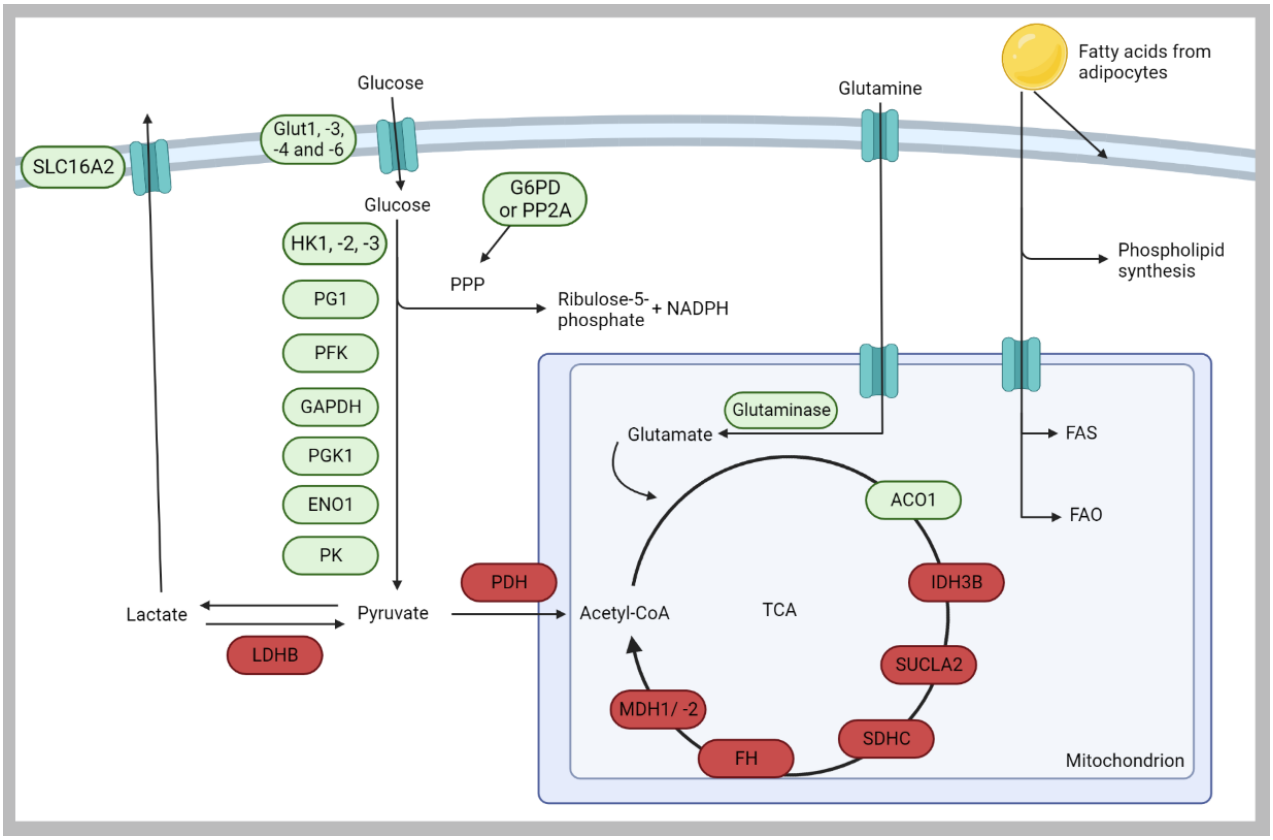

Figure 2. *Proteins and enzymes of glycolysis and the citric acid cycle (TCA). Proteins highlighted in green often found upregulated and proteins highlighted in red often downregulated in B-ALL. Many glycolytic enzymes show a higher level of expression in B-ALL explaining the highly glycolytic phenotype. Also, the enzymes catalyzing reaction from lactate to pyruvate and pyruvate to acetyl-CoA were found downregulated. These explain the Warburg effect which is apparent in B-ALL. Aconitase 1 (ACO1), enolase 1 (ENO1), fatty acid oxidation (FAO), fatty acid synthesis (FAS), fumarase (FH), glucose-6-phosphate dehydrogenase (G6PD), glyceraldehyde-3-phoshpate dehydrogenase (GAPDH), glucose transporter (Glut), hexokinase (HK), isocitrate dehydrogenase (IDH3B), lactate dehydrogenase B (LDHB), malate dehydrogenase (MDH), pyruvate dehydrogenase (PDH), phosphofructokinase (PFK), glucose-6-phosphate isomerase (PGI), phosphoglycerate kinase 1 (PGK1), pyruvate kinase (PK), threonine protein phosphatase 2A (PP2A), pentose phosphate pathway (PPP), succinate dehydrogenase complex subunit C (SDHC), solute carrier family 16 member 2 (SLC16A2), succinyl-CoA ligase (SUCLA2). Image created with BioRender.com.*

MYC and *RAS* drive metabolic rewiring. Oncogenic Myc expression promotes glycolysis, glutaminolysis, mitochondrial biogenesis, lipid synthesis and nucleotide biosynthesis. Oncogenic *RAS* mutations on the other hand also promote glutaminolysis and increased glucose uptake as well as

causing shift towards more anabolic metabolism (Rashkovan and Ferrando, 2019, Soltani *et al.* 2021).

BCR-ABL is a fusion gene which acts as an oncogene in B-ALL (Malard and Mohty, 2020). It is more commonly found in B-ALL adult patients and is often associated with *IKZF1* mutations (Malard and Mohty, 2020). The *BCR-ABL1* transgene alone does not cause an increase in ATP levels and glucose uptake of lymphocytes significant enough to cause a malignant transformation but has shown to correlate with a poor prognosis B-ALL (Chan *et al.* 2017, Malard and Mohty, 2020). By restricting cellular ATP levels to which are inadequate for leukemogenesis and malignant transformation, PAX5 and IKZF1 act as metabolic gatekeepers (Chan *et al.* 2017). The tumor suppression function of them involves a transcriptional repression of glucose transport and metabolites to TCA (Chan *et al.* 2017). In addition to enabling glucose uptake and glycolysis, PAX5, IKZF1, EBF1, and TCF3, also show binding peaks at promoter areas of *NR3C1*, *TXNIP* and *CNR2* – the negative regulators of glucose uptake (Chan *et al.* 2017). Disrupting mutations to *PAX5* lead to reduced expression of *NR3C1*, a glucocorticoid receptor (GR) which is linked to reduced sensitivity towards glucocorticoids (GC) (Chan *et al.* 2017, Sbirkov *et al.* 2020). Pax5 and IKZF1 maintain pre-B cells normal metabolism preventing malignant transformation by limiting the amount of available glucose and cellular ATP (Chan *et al.* 2017). Mutations in *PAX5* occur in over 30 % of B-ALL cases (Mullighan *et al.* 2007), however, in B-ALL with *BCR-ABL1,* mutations of *PAX5* and *IKZF1* genes are present over 80 % of the time (Chan *et al.* 2017). Negative mutations to *PAX5* or *IKZF1* release glucose and energy restrictions (Chan *et al.* 2017, Martín-Lorenzo *et al.* 2018). Wild-type Pax5 binds to promoter regions of many glycolytic enzymes and key metabolic regulators (*ACO1, ENO1, G6PC3, G6PD, GAPDH, HK2/ -3, IDH1, MYC1, PGAM, PGK1,* and *PYGL*) acting a repressor (Martín-Lorenzo *et al.* 2018). Deletion of *PAX5* in *BCR-ABL1* positive mouse pre-B ALL models resulted in a more than 25-fold increase in glucose uptake and ATP levels (Chan *et al.* 2017). Martín-Lorenzo, A. et al. found similar results in their study where they tackled the question, what causes the conversion of *BCR-ABL1* pre-leukemic cells into pre-B-ALL cells. A loss of Pax5 increased total ATP levels by 3-fold in non-leukemic cells and a *BCR-ABL1* fusion gene in non-leukemic cells increased ATP levels also by 3 times compared to normal cell line counterparts (Martín-Lorenzo *et al.* 2018). However, in *BCR-ABL1* harboring Pax5-deficient leukemic cells glucose consumed, lactate produced and total ATP were drastically increased (55-times, 37-times, and 16-times respectively) (Martín-Lorenzo *et al.* 2018). In these leukemic cells increases in mitochondrial ATP production was also observed (Martín-Lorenzo *et al.* 2018). Both Martín-Lorenzo *et al.* (2018) and Chan *et al.* (2017) concluded that loss of Pax5 or IKZF1 drives leukemogenesis trough metabolic reprogramming.

In B-ALL and T-ALL, AMPK, an energy stress sensor, has been shown to be activated (Kishton *et al.* 2016, Chan *et al.* 2017). AMPK is activated trough increased levels of AMP and alleviates energy stress trough inhibition of cells energy consuming processes while stimulating energy production. Levels of glucose, pyruvate and ATP are substantially lower in B-ALL than in acute myeloid leukemia (AML) cells (Chan *et al.* 2017). This chronic energy stress led to the activation of the LKB1-AMPK which stimulates catabolic metabolism while simultaneously inhibiting anabolic metabolism to increase energy levels in cells (Pearce *et al.* 2013, Chan *et al.* 2017). AMPK directly suppresses mTORC1 (Pearce *et al.* 2013, Kishton *et al.* 2016). However, also functional Pax5 and IKZF1 can reduce glucose and ATP levels in B-ALL, leading to a state of energy stress and resultant AMPK activation (Chan *et al.* 2017). Interestingly high expression of LKB1 and AMPK were linked to poor clinical outcomes (Chan *et al.* 2017).

Targeting AMPK has been found to be an effective treatment for B-ALL (Chan *et al.* 2017). Loss of LKB1 or AMPKα2 (a isoform of AMPK) in B-ALL cells decreased glucose uptake, mitochondrial respiration and ATP levels leading to rapid leukemia cell death while prolonging survival of mouse recipients and delayed leukemia onset (Chan *et al.* 2017).

Cancer cells require lipids and fatty acids for proliferation (DeBerardinis and Chandel, 2016). Upregulation of lipogenic enzymes such as ACC1 and FASN have been observed in many cancers and it correlates with a poor prognosis and as such are promising targets for cancer treatment (Tucci *et al.* 2021). However, in a recent study B-ALL cells were observed to induce free fatty acid secretion from adipocytes (Tucci *et al.* 2021). These fatty acids are taken up by ALL cells and can be incorporated into metabolism or cell membranes (Tucci *et al.* 2021). B-ALL cells near adipocytes exhibited lower levels of lipogenic enzymes (ACC1, FASN, SCD1) indicating a lowered dependency on fatty acid metabolism (Tucci *et al.* 2021). Increased levels of adipocyte-derived fatty acids were linked to increased therapy resistance which could explain worse outcomes experienced by obese patients (Tucci *et al.* 2021).

3.2 Metabolism in T-ALL cells

Although high glycolysis is a conservated feature in ALL cells, T-ALL cells possess a less glycolytic, and a more oxidative metabolic phenotype compared to B-ALL cells (Kishton *et al.* 2016, Mirabilii *et al.* 2020). A common characteristic in T-ALL is an activated NOTCH pathway (Herranz *et al.* 2015, Sbirkov *et al.* 2020). Activation in the pathway occurs in more than 60% of patients and oncogenic NOTCH signaling can also activate the PI3K-Akt pathway (Herranz *et al.* 2015, Kishton *et al.* 2016). Activation of PI3K-Akt pathway is found in 81% of T-ALL samples (Sanchez *et al.* 2019). The metabolic changes observed in T-ALL can be attributed, in part, to the interplay between two cellular signaling pathways: mTOR and AMPK (Kishton et al. 2016). Specifically, mTOR can be activated through various mechanisms, including the NOTCH and PI3K-Akt pathways. Upon

activation, these pathways drive an increase in metabolic capacity by promoting changes in metabolism that favor more active anabolic pathways and glycolysis (Kishton *et al.* 2016; Sbirkov *et al.* 2020, Kumar *et al.* 2022) (figure 1). PTEN is the main negative regulator of PI3K-Akt pathway and is inactivated in 35% of patients (Rashkovan and Ferrando, 2019, Sbirkov *et al.* 2020). Combination of NOTCH and PI3K-Akt signaling with loss of PTEN results in increased aerobic glycolysis (Herranz *et al.* 2015, Rashkovan and Ferrando, 2019). This is consistent with the results of Daniel Herranz's group who reported that loss of PTEN or activated Akt increased cellular lactate levels (Herranz *et al.* 2015). However the group also observed that TCA was prominently fueled by glutaminolysis in NOTCH1-induced leukemias with wild-type *PTEN* (Herranz *et al.* 2015). Additionally, T-ALL with functioning PTEN showed a much less glycolytic phenotype while being more reliant on glutaminolysis compared to PTEN-deficient counterparts (Herranz *et al.* 2015). Metabolic isotope labeling analyses showed 80 % of the carbon in TCA originating from glutamine (Herranz *et al.* 2015). Inhibition of NOTCH1 resulted in decrease in glycolysis and glutaminolysis, accumulation of glycolytic intermediates, and signs of autophagy in T-ALL with functional PTEN (Herranz *et al.* 2015). These results suggest NOTCH1-induced T-ALL cells implement active aerobic glycolysis while fueling mitochondrial metabolism trough glutaminolysis.

The anabolically demanding phenotype of T-ALL is not energetically sustainable and causes elevated energy stress levels in T-ALL cells and the consequent AMPK activation (Kishton *et al.* 2016). A study conducted with primary murine T-cell acute lymphoblastic leukemia cells revealed that T-ALL cells experience chronic ATP insufficiency, which in turn promotes AMPK activity. AMPK actively restrains aerobic glycolysis through strict inhibition of mTORC1 and by upregulation of mitochondrial Complex I, promoting oxidative metabolism while inhibiting anabolic metabolism (Pearce *et al.* 2013, Kishton *et al.* 2016). By upregulation of mitochondrial activity, reducing energy demand and promoting energy generation, AMPK increases cancer cell survival (Kishton *et al.* 2016). AMPK inhibits fatty acid, protein, and glycogen synthesis (Rashkovan and Ferrando, 2019). Contradictory to this, Rashkovan and Ferrando (2019) stated that instead of AMPK being an inhibitor of glycolysis, its activation would instead upregulate it. Other sources indicate to AMPK acting as an inhibitor of glycolysis and anabolic metabolism (Pearce *et al.* 2013).

Global analysis of T-ALL metabolomics found that T-ALL cells to have persistently elevated glucose uptake, glycolytic activity, and PPP flux compared to naïve T cells (Kishton *et al.* 2016). Surprisingly though these all were significantly lower than in activated T-cells (Kishton *et al.* 2016). Activated T-cells can utilize PI3K-Akt pathway and Myc upon activation to rapidly proliferate resulting in a much higher energy production (Pearce *et al.* 2013, Kishton *et al.* 2016). T-cells stimulated for 48h showed tremendous increase in glycolytic rate and glucose uptake whereas T-ALL cells had increased concentrations of metabolites in FAO and amino acid oxidation with their mitochondrial content and potential being similar (Kishton *et al.* 2016). Similar AMPK activity is found in both

stimulated T cells and T-ALL cells, but its targets were different (Kishton *et al.* 2016). Activated healthy T-cells had much lower amounts of pS6 and pEBP1 (proteins involved in cell growth and proliferation) compared to T-ALL cells (Kishton *et al.* 2016). T-ALL cells showed phosphorylation of Raptor which is observed in naïve T-cells but not in activated T-cells (Kishton *et al.* 2016). Raptor (subunit of mTOR complex 1) phosphorylation is linked to lower levels of glycolysis and mTORc1 activity (Kishton *et al.* 2016).

3.3 ALL Relapse and Resistance to Therapy

Even with the advances in childhood ALL treatment, approximately 10 % of patients experience relapse and the number is higher in adults and older patients (Malard and Mohty, 2020, Li *et al.* 2021). One reason is an acquired resistance to GCs and it is one of the key predictors of relapse in ALL (Buentke *et al.* 2011, Li *et al.* 2021). There is yet no comprehensive explanation to the mechanism behind GC-resistance in ALL but rather multiple speculations of factors which contribute to the development of GC-resistance (Hulleman *et al.* 2009, Dobson *et al.* 2020, Li *et al.* 2021). One such phenotype correlating with GC-resistance is activity of glycolysis (Hulleman *et al.* 2009, Buentke *et al.* 2011, Rashkovan and Ferrando, 2019).

GCs reduce glucose metabolism of ALL cells and increased glycolytic activity correlates with GC-resistance (Hulleman *et al.* 2009, Buentke *et al.* 2011). Thus, inhibiting glycolysis has proven to reverse resistances to GCs dexamethasone and prednisolone (Hulleman *et al.* 2009, Buentke *et al.* 2011). Myc and HIF1α are thought to be key components in the differing gene expression levels of GC-resistant and -sensitive ALL cells (Hulleman *et al.* 2009). Also HIF1α directly influences glucose consumption (Mirabilii *et al.* 2020). However, silencing of HIF1α by RNA interference did not affect cell lines sensitivity towards GCs (Hulleman *et al.* 2009). Akt and mTOR inhibition were shown to sensitize ALL cells to GCs (Hulleman *et al.* 2009). GR loss is and obvious explanation to GC-resistance (Dobson *et al.* 2020, Li *et al.* 2021). However, high levels of GR are also observed in many GC-resistant cell lines proving that GC-resistance is a more complicated matter than merely changes in GR expression (Li *et al.* 2021).

Characterization of 14 patient derived B-ALL samples were further cultured in mice recipients, purified, and classified into three categories: ALL cells found at diagnosis, partially evolved subclones initiating relapse and drug tolerant relapse samples of ALL. Gene expression and whole genome sequencing analysis revealed relapse initiating clones (dRI) exhibiting upregulated mitochondrial, cellular, amino acid and lipid metabolism. Moreover, increased levels of mTOR and total mitochondrial mass were found in dRI and relapse clones. Many pathways enriched in dRI clones were even more active in relapse clones. Uniquely upregulated pathways in relapse clones were mainly associated with cell cycle regulation. (Dobson *et al.* 2020)

Another study characterizing a novel GC-resistant B-ALL cell line found many contradictory results to previous studies. In summary, the GC-resistant cell line of Li *et al.* had a relatively low glycolytic phenotype, low rates of FAS, downregulated AMPK activity as well as inhibition in mTOR pathway, suggesting AMPK and glycolysis are poor therapeutic targets in GC-resistant ALL (Li *et al.* 2021). Increases in FAS activity and glycolytic rates are general markers of poor prognosis in many cancers (Li *et al.* 2021, Grüninger *et al.* 2022) and increases in glycolytic rates directly correlated with GC-resistance (Hulleman *et al.* 2009, Buentke *et al.* 2011). The study by Li et al. (2021) found that in GC-resistant cell lines showed reduced expression of important enzymes involved in glycolysis and FAS. Interestingly it contradicts previous findings on the characteristics of GC-resistant B-ALL cell lines. Suggesting there are multiple subtypes of GC-resistant B-ALL cells, each with its own distinct set of features and drug tolerance mechanisms, which arise from complex interplays among various biological pathways.

4. DRUG TARGETING

Metabolic reprogramming brings essential advantages to cancer cells enabling their biomass accumulation and proliferation (DeBerardinis and Chandel, 2016, Kumar *et al.* 2022). Thus, targeting the distinct metabolic features of malignant cells has become a promising method of treatment. Inhibiting metabolic pathways, essential for cancer cell survival, show anti-tumor effects varying from reduced tumor mass to accumulation metabolites and to apoptosis.

GCs have been used in the treatment of ALL for over 60 years and still remain an integral part in the treatment of ALL (Buentke *et al.* 2011, Malard and Mohty, 2020). Most common GCs used in B-ALL treatment are prednisolone and dexamethasone (Hulleman *et al.* 2009, Buentke *et al.* 2011). Buentke et al. were the first to show dexamethasone targeting and fundamentally altering ALL cellular metabolism by inhibiting its glucose uptake and processing (Buentke *et al.* 2011). The level of reduction in glycolytic rates correlated with increased cell-cycle arrest and induction of cell death (Buentke *et al.* 2011). Though higher expression of glycolytic enzymes is associated with GC-resistance, this resistance has been shown to be reversable through glycolysis inhibition with drugs such as 2DG, lonidamine and 3-bromopyruvate (Hulleman *et al.* 2009, Buentke *et al.* 2011, Rashkovan and Ferrando, 2019). In addition to relieving GC-resistance in ALL cells, 2DG has shown strong synergistic effects with GCs in treatment of GC-resistant cells (Hulleman *et al.* 2009). Though this synergistic effect is only found in resistant cell lines and not in GC-sensitive cells (Hulleman *et al.* 2009). In addition to reversing GC-resistance, non-cytotoxic inhibition of glucose

uptake and glycolysis was shown to sensitize cells and increase efficacy to other therapies such as tyrosine kinase inhibitor Dasatinib (Liu *et al.* 2014, Rashkovan and Ferrando, 2019).

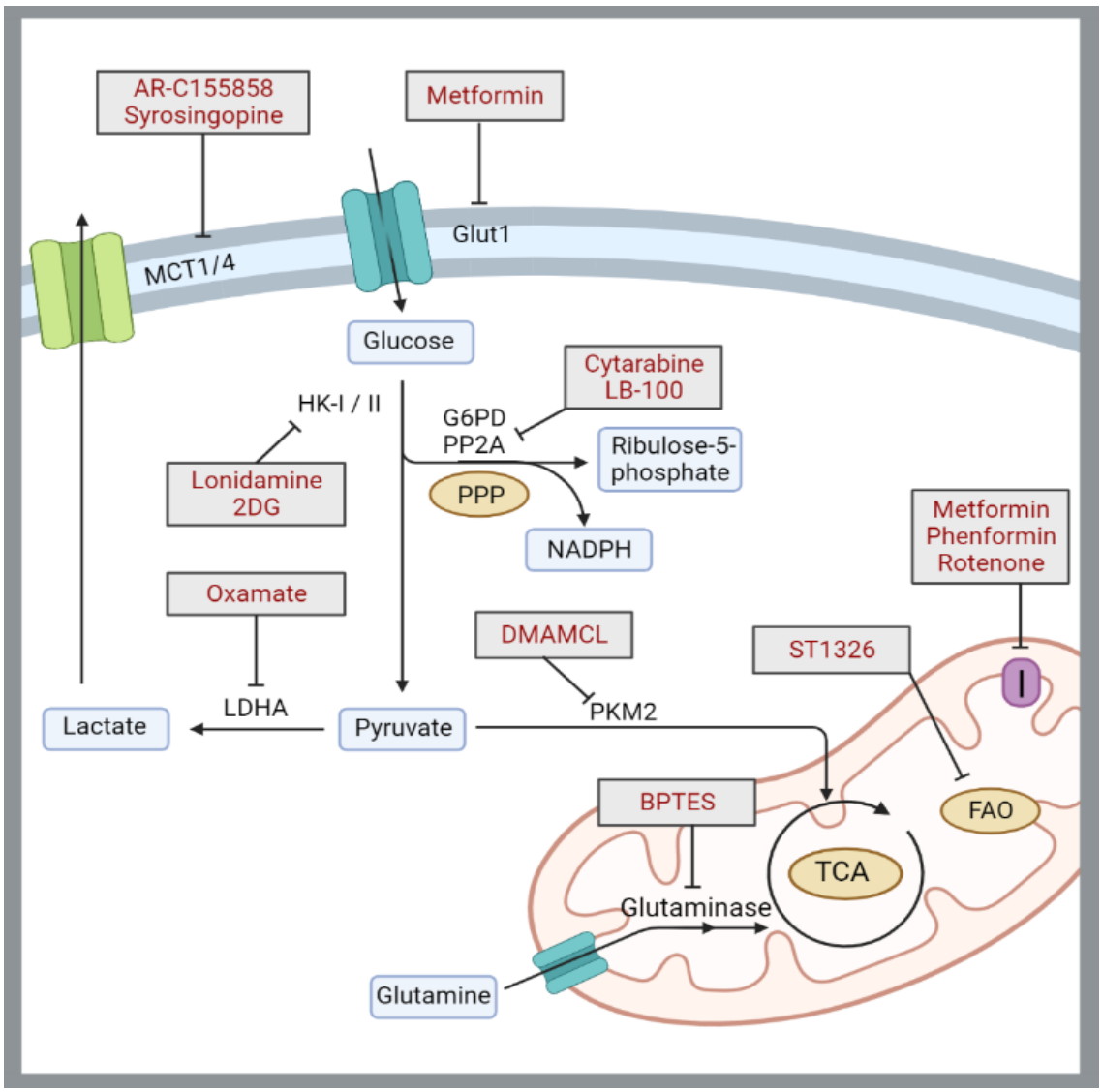

Figure 3. *Drugs directly targeting cellular energy metabolism. These drugs have shown promising results in ALL preclinical studies and studies of other subtypes of leukemia. Fatty acid oxidation (FAO), glucose-6-phosphate dehydrogenase (G6PD), glucose transporter (Glut), hexokinase (HK), mitochondrial Complex I (I), lactate dehydrogenase (LDHA), monocarboxylate transporter (MCT), pyruvate kinase M2 (PKM2), threonine protein phosphatase 2A (PP2A), the citric acid cycle (TCA). Image created with BioRender.com.*

Aerobic glycolysis is the dominant way of energy production in B- and T-ALL cells and most glucose consumed is catabolized trough glycolysis (Kishton *et al.* 2016, Sbirkov *et al.* 2020, Soltani *et al.* 2021). This suggests that targeting of glycolysis and glucose uptake can be an effective strategy in ALL treatment. Glucose transporters Glut1, -3, -4 and -6 are often upregulated in ALL, especially Glut1 and -4 (Boag *et al.* 2006, Kishton *et al.* 2016, Martín-Lorenzo *et al.* 2018, Mirabilii *et al.* 2020). Glut4 transporter inhibition with metformin has proven an effective target in chronic lymphoblastic

leukemia (CLL) (Soltani *et al.* 2021). Moreover, in B-ALL *Glut1* deletion showed decreased proliferation and suppression of leukemia progression (Liu *et al.* 2014). In addition, the high rate of aerobic glycolysis creates a high lactate and high hydrogen ion concentration microenvironment in the bone marrow (Soltani *et al.* 2021). This environment was found to be beneficial to ALL cells via the impairment of immune function (Soltani *et al.* 2021). Neutralization of this acidic environment may aid in the treatment of ALL (Soltani *et al.* 2021).

Rate limiting enzymes of glucose utilization, such as G6PD, HK, lactate dehydrogenase (LDHA), PKM2, and GAPDH are other promising targets for therapies (Hulleman *et al.* 2009, Soltani *et al.* 2021). LDHA inhibition by oxamate stops ALL cell cycle into G0/G1-phase inducing apoptosis and also inactivates Myc and Akt signaling (Soltani *et al.* 2021). The last step of aerobic glycolysis, secretion of lactate and hydrogen ions, is also a potential target. Monocarboxylate transporter (MCT) inhibition causes accumulation of lactate, intracellular acidification, and apoptosis (Soltani *et al.* 2021). AR-C155858 and syrosingophine (inhibitors of MCT1 and MCT4 respectively) have been studied in AML cells exhibiting antiproliferative and pro-apoptotic effects also synergizing with standard chemotherapy agents (Soltani *et al.* 2021).

PPP provides metabolites for nucleotide biosynthesis and maintains NADPH production to protect cells from oxidative stress (DeBerardinis and Chandel, 2016, Rashkovan and Ferrando, 2019). In B-ALL cells with functional Pax5 and IKZF1, G6PD, a rate limiting enzyme transferring glucose into PPP, is usually downregulated (Chan *et al.* 2017, Martín-Lorenzo *et al.* 2018, Rashkovan and Ferrando, 2019). In B-ALL threonine protein phosphatase 2A (PP2A), which functions in redirecting glucose into PPP, is often upregulated which balances the reduced rate of PPP (Rashkovan and Ferrando, 2019). LB-100 inhibits PP2A ceasing glucose flux into PPP (Rashkovan and Ferrando, 2019). Effect of this shunt in PPP activity has antitumor effects resulted from oxidative stress (Rashkovan and Ferrando, 2019). Similarly to PP2A inhibition, G6PD inhibition, might provide beneficial effects in therapy of B- and T-ALL. G6PD inhibition in AML with cytarabine provides anti-leukemic effects (Soltani *et al.* 2021).

mTOR inhibition has provided interesting results for B-ALL treatment. mTOR and Akt phosphorylation (inhibition) relieves GC-resistance (Hulleman *et al.* 2009). Moreover, both mTOR inhibition by everolimus and Akt inhibition with capivasertib led to reduced lipogenesis, protein synthesis and glycolytic rates (Grüninger *et al.* 2022). Treatments also resulted in a metabolic shift toward beta-oxidation, glutamine utilization and an increased dependency on them (Grüninger *et al.* 2022). mTOR inhibition could have potential synergistic effects with simultaneous FAO or other mitochondrial metabolism inhibition and could play a part in treatment of GC-resistant ALL. AMPK activity – a direct inhibitor of mTOR – correlates with poor prognosis and increased survival of ALL cells (Kishton *et al.* 2016, Chan *et al.* 2017). mTOR inhibition could lead to increased cancer cell survival trough similar functions as AMPK activity.

FAO inhibition has provided good results in treatment of other types of leukemias (AML and CLL) (Soltani *et al.* 2021). Although it is important to note the differences in these subtypes' metabolisms. CLL is more reliant of energy produced from FAO whereas it is usually suppressed in B-ALL (Boag *et al.* 2006, Sbirkov *et al.* 2020, Soltani *et al.* 2021). In CLL, inhibition of CPT's (a transporter of fatty acids from cytosol into mitochondria) lead to substantial cell death rates (Soltani *et al.* 2021). Similarly in AML ST1326, an inhibitor of CPT, caused mitochondrial damage, reduced proliferation, growth arrest, and apoptosis (Rashkovan and Ferrando, 2019). Fatty acid metabolism can be also targeted trough FASN inhibition. FASN functions in converting malonyl CoA into fatty acid chains (Soltani *et al.* 2021, Tucci *et al.* 2021). Elevation in FASN levels has been observed in drug resistant ALLs correlating with a poor prognosis (Soltani *et al.* 2021). FASN inhibition reversed dexamethasone resistance simultaneously inducing apoptosis in ALL (Soltani *et al.* 2021). However, adipocytes have been shown to provide fatty acids to ALL cells which was linked to increased therapy resistance (Tucci *et al.* 2021). By inhibiting the secretion of lipids from adipocytes or suppressing the signals that induce it, better treatment outcomes could be achieved in ALL, particularly in obese patients.

Lai N. Chan et al. identified TXNIP, CNR2 and AMPK as novel targets in pre-B ALL treatment (Chan *et al.* 2017). Loss of LKB1 or AMPKα2 reduced B-ALL viability and survival drastically (Chan *et al.* 2017). BML275 suppressed AMPK activity causing, energy stress, AMP accumulation and cell death (Chan *et al.* 2017). In B-ALL treatment, BML275 showed strong synergistic effects with prednisolone (Chan *et al.* 2017). Moreover, in T-ALL, AMPK plays an integral part in the stabilization of cells metabolic phenotype and the deletion of AMPKα1 led to reduced T-ALL cell numbers and correlated with increased animal survival (Kishton *et al.* 2016). T-ALL cells are more reliant on mitochondrial metabolism than aerobic glycolysis for energy production (Kishton *et al.* 2016). Metformin, rotenone and phenformin – inhibitors of mitochondrial Complex I – can rapidly induce cell death of T-ALL cells *in vitro* as well as *in vivo* by triggering energy stress and autophagy (Kishton *et al.* 2016, Rashkovan and Ferrando, 2019). Low doses of rotenone and phenformin were found to rapidly kill primary T-All cells while sparing and significantly not altering viability *ex vivo* T-cells and stimulated T-cells (Kishton *et al.* 2016). Effects of phenformin were further tested *in vivo* by Kishton R. et al. and they found mouse T-ALL cells number and percentage much lower than in vehicle treated mice (Kishton *et al.* 2016). Phenformin treatment increased mice survival (Kishton *et al.* 2016). Metformin (a similar molecule to phenformin) does not only have individual benefits in T-ALL treatment but in addition has shown synergizing effects with drugs commonly used in leukemia treatment – vincristine, and L-asparaginase (Rashkovan and Ferrando, 2019, Malard and Mohty, 2020).

Commonly activated NOTCH pathway in T-ALL causes upregulation of glycolysis, glutaminolysis, anabolic metabolism and the resultant AMPK activation (Kishton *et al.* 2016). Inhibition of NOTCH1

results in reduced activities of these and autophagy (Herranz *et al.* 2015). Glutaminolysis is an essential feature in T-ALL and BPTES, a small molecule inhibitor of glutaminase, impairs T-ALL cell growth and was shown to strongly synergize with coincident NOTCH1 inhibition with DBZ (Herranz *et al.* 2015). This synergistic antitumor effect of BPTES and DBZ was replicated in *PTEN*-positive T-ALL treatment in mice (Herranz *et al.* 2015). However, *PTEN*-deleted T-ALL cells were more resistant towards this treatment indicating a more glycolytic phenotype (Herranz *et al.* 2015). In *PTEN*-deleted T-ALL treatment, targeting glycolysis could provide desirable results.

5. CONCLUSION

Regulatory pathways of metabolism in leukemic cells are largely interconnected providing a complex entity all influencing the resultant phenotype (figure 1). Metabolic reprogramming has been connected to increased cancer cell growth as well as treatment resistance. The apprehension of this intricate system can provide new methods and targets for treatments of ALL such as the identification of the essential role of AMPK in ALL (Kishton *et al.* 2016, Chan *et al.* 2017).

Active aerobic glycolysis is a conserved feature in both B- and T-ALL although the level of it varies (Boag *et al.* 2006, Kishton *et al.* 2016, Chan *et al.* 2017). Contrary to B-ALL, T-ALL cells are not as highly dependent on energy produced from glycolysis, rather on mitochondrial metabolism (Kishton *et al.* 2016, Mirabilii *et al.* 2020). This is from the result of energy stress caused by over demand of anabolic metabolism resulting in the energy stress sensor AMPK activation balancing the energy consumption to generation (Kishton *et al.* 2016). This phenomenon is also present in B-ALL (Chan *et al.* 2017). Transcription factors Pax5 and IKZF1 function as gatekeepers of metabolic reprogramming in B-ALL cells (Chan *et al.* 2017, Martín-Lorenzo *et al.* 2018). In B-ALL, the existence *of BCR-ABL1* transgene is not enough to confer metabolism to the extent of allowing malignant transformation (Chan *et al.* 2017, Martín-Lorenzo *et al.* 2018). However, loss of function mutations or deletion of *PAX5* and *IKZF1* absolves the inhibition of glycolysis in *BCR-ABL1* positive cells and has shown increases in ATP levels by more than 25-times (Chan *et al.* 2017).

Overall, the findings presented in this review indicate that metabolic reprogramming plays a crucial role in the pathogenesis of ALL. The altered metabolism of leukemic cells results in their enhanced proliferation, survival, as well as resistance to chemotherapy. The alterations have been observed to happen at any point from leukemogenesis to relapse. Therefore, targeting the metabolic pathways that are dysregulated in ALL could represent a promising approach for the development of more effective therapies. The metabolic alterations occurring in ALL and other cancers provide a promising avenue for therapeutic intervention all the way from increasing efficacy of treatment to

reversing drug resistance. Synergistic effects of inhibition of multiple pathways or alongside standard chemotherapy drugs suggest that drugs targeting metabolism of ALL cells could play a role in improving outcomes. A better understanding of the mechanisms underlying metabolic reprogramming in leukemia cells could facilitate the development of more effective treatments that exploit the metabolic vulnerabilities of ALL cells.

REFERENCES

Boag J.M., Beesley A. H., Firth M.J., *et al.* (2006). Altered glucose metabolism in childhood pre-B acute lymphoblastic leukaemia. *Leukemia* 20(10):1731–1737.

Buentke E., Nordström A., Lin H., *et al.* (2011). Glucocorticoid-induced cell death is mediated through reduced glucose metabolism in lymphoid leukemia cells. *Blood Cancer Journal* 1(7):e31.

Chan L.N., Chen Z., Braas D., *et al.* (2017). Metabolic gatekeeper function of B-lymphoid transcription factors. *Nature* 542(7642):479–483.

DeBerardinis R.J. and Chandel N.S. (2016). Fundamentals of cancer metabolism. *Science Advances* 2(5):e1600200.

Dobson S.M., Garcia-Prat L., Vanner R.J. *et al.* (2020). Relapse-Fated Latent Diagnosis Subclones in Acute B Lineage Leukemia Are Drug Tolerant and Possess Distinct Metabolic Programs. *Cancer Discovery* 10(4):568–587.

Grüninger P.K., Uhl F., Herzog H., *et al.* (2022). Functional characterization of the PI3K/AKT/MTOR signaling pathway for targeted therapy in B-precursor acute lymphoblastic leukemia. *Cancer Gene Therapy* 29(11):1751–1760.

Gu Z., Chirchman M.L., Roberts K., *et al.* (2019). PAX5-driven subtypes of B-progenitor acute lymphoblastic leukemia. *Nature Genetics* 51(2):296–307.

Herranz D., Ambesi-Impiombato A., Sudderth J., *et al.* (2015). Metabolic reprogramming induces resistance to anti-NOTCH1 therapies in T cell acute lymphoblastic leukemia. *Nature Medicine* 21(10):1182–1189.

Hulleman E., Kazemier K.M., Holleman A., *et al.* (2009). Inhibition of glycolysis modulates prednisolone resistance in acute lymphoblastic leukemia cells. *Blood* 113(9):2014–2021.

Kishton R.J., Barnes C.E., Nicholas A.G., *et al.* (2016). AMPK Is Essential to Balance Glycolysis and Mitochondrial Metabolism to Control T-ALL Cell Stress and Survival. *Cell Metabolism* 23(4):649–662.

Kumar R., Mishra A., Gautam P. *et al.* (2022). Metabolic Pathways, Enzymes, and Metabolites: Opportunities in Cancer Therapy. *Cancers* 14(21):5268.

Li Y., Zuo C. and Gu L. (2021). Characterization of a novel glucocorticoid-resistant human B-cell acute lymphoblastic leukemia cell line, with AMPK, mTOR and fatty acid synthesis pathway inhibition. *Cancer Cell International* 21(1):623.

Liu T., Kishton R.J., Macintyre A.N., *et al.* (2014). Glucose transporter 1-mediated glucose uptake is limiting for B-cell acute lymphoblastic leukemia anabolic metabolism and resistance to apoptosis. *Cell Death & Disease* 5(10):e1470–e1470.

Malard F. and Mohty M. (2020). Acute lymphoblastic leukaemia. *Lancet (London, England)* 395(10230):1146–1162.

Martín-Lorenzo A., Auer F., Chan L.N., *et al.* (2018). Loss of Pax5 exploits Sca1-BCR-ABLp190 susceptibility to confer the metabolic shift essential for pB-ALL. *Cancer research* 78(10):2669–2679.

Mirabilii S., Ricciardi M.R. and Tafuri A. (2020). mTOR Regulation of Metabolism in Hematologic Malignancies. *Cells* 9(2):404.

Mullighan C.G., Goorha S., Radtke I., *et al.* (2007). Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* 446(7137):758–764.

Pearce E.L., Poffenberger M.C., Chang C.H., *et al.* (2013). Fueling immunity: insights into metabolism and lymphocyte function. *Science (New York, N.Y.)* 342(6155):1242454.

Rashkovan M. and Ferrando A. (2019). Metabolic dependencies and vulnerabilities in leukemia. *Genes & Development* 33(21–22):1460–1474.

Sanchez V.E., Nichols C., Kim H.N., *et al.* (2019). Targeting PI3K Signaling in Acute Lymphoblastic Leukemia. *International Journal of Molecular Sciences* 20(2):412.

Sbirkov Y., Burnusuzov H. and Sarafian V. (2020). Metabolic reprogramming in childhood acute lymphoblastic leukemia. *Pediatric Blood & Cancer* 67(6):e28255.

Soltani M., Zhao Y., Xia Z., *et al.* (2021). The Importance of Cellular Metabolic Pathways in Pathogenesis and Selective Treatments of Hematological Malignancies. *Frontiers in Oncology* 11.

Tucci J., Chen T., Margulis K., *et al.* (2021). Adipocytes Provide Fatty Acids to Acute Lymphoblastic Leukemia Cells. *Frontiers in Oncology* 11:665763.

Warburg O., Wind, F. and Negelein, E. (1927). The Metabolism of Tumors in the Body. *The Journal of General Physiology* 8(6):519–530.