Book Chapter

Metabolic Deregulations in Acute Myeloid Leukemia

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Introduction

Acute myeloid leukemia (AML) is a bone marrow cancer affecting the myeloïd compartment of hematopoietic cells, leading to the accumulation of immature cells called leukemic blasts in the bone marrow and the peripheral blood. The leukemic population comprises stem cells and cells at a very

early stage of differentiation. Consequently, the prognosis of AML is very poor, with a 5-year survival rate(excluding AML3)of about 35-40% for patients until 60 years, and decreasing to 5-15% for patients over 60 years of age [1].

Molecular genetic, abnormalities associated with the emergence and development of leukemic clones, revealed that many cellular functions are deregulated [2]. Mutations occurring in the *FLT3*, *RAS* or *KIT* genes result in hyperactivation of signaling pathways [3,4] providing a proliferative advantage to the transformed cells. Oncogenic fusion genes, such as *PML-RARA* or *AML1-ETO* [5] are responsible for cell differentiation blockage, and mutations in genes involved in epigenetic control of gene expression, such as *ASXL1*, *DNMT3A*, *IDH* or *TET2*, lead to deregulation of the chromatin structure (via chemical changes in histones) or abnormalities in DNA methylation [1]. Finally, it is now well documented that these abnormalities can also lead to alterations in metabolism that play a critical role in leukemogenesis and in the phenotypic characteristics of leukemic cells [6]. The most described example in AML are the *IDH1* and *IDH2* mutations, which lead to production of an oncometabolite, the 2-Hydroxy-Glutarate (2HG), instead of α-Ketoglutarate (α-KG) [7]. The increase in 2-HG directly impacts the metabolic functions of leukemic cells promoting their proliferation at the expense of normal hematopoietic stem cells (see below for more details of impact of *IDH* mutations).

I-Metabolic Deregulation in AML

Like most cancer cells, AML cells are characterized by a rapid and uncontrolled proliferation that requires nutrients and energy. To achieve this, several mechanisms are put into play. The best known is the Warburg effect, described in the 1920s by Otto Heinrich Warburg, who showed that regardless of oxygenation conditions, cancer cells preferentially use aerobic glycolysis, which converts glucose into lactate, to produce energy and maintain cancer proliferation [8]. Despite poor energy efficiency (in terms of the production of ATP molecules), cancer cells maintain aerobic glycolysis to favor the production of intermediate products from glycolysis, mandatory for generating

nucleotides, lipids or amino acids. This allows a rapid supply of the components essential to the formation of future daughter cells, and thus a more effective cell proliferation [9]. Beyond the Warburg effect, many metabolic pathways are involved in the leukemic process.

Mutations of Genes Encoding the Isocitrate Dehydrogenase (IDH)

Isocitrate dehydrogenase (IDH) catalyzes the decarboxylation of isocitrate leading to the production of alpha-ketoglutarate (KG) and NAdPH/NADH. IDH1, located in the cytoplasm, promotes the generation of NADPH but also, thanks to the carboxylation of the α-KG in isocitrate, the acetylCoA and lipid generation after conversion of citrate into acetyl-coA. IDH2 regulates the concentration of α-KG and isocitrate in the mitochondria using NAD to oxidize isocitrate in α-KG in the Tri-carboxylic acid (TCA) cycle [10]. Finally, there is a third isoform, the IDH3, which is NAD-dependent and which promotes the production of α-KG required for the generation of succinate and then ATP [11].

In AML, *IDH* gene mutations are found in about 15-20% of patients. Only the two isoforms IDH1 and IDH2 can be mutated but the two mutations are mutually exclusive. Mutations affect arginine residues critical for the IDH function, codon R132 for *IDH1* and codons R140 and R172 for *IDH2*. They are "gain of function" mutations that both result in the production of a new metabolite, the 2-Hydroxy-Glutarate (2HG) instead of KG in tumor cells [7]. It should be noted that the mutation does not cause any change in the concentration of α-KG in the serum, nor other intermediary metabolites of the TCA cycle, such as succinate, malate or fumarate [12]. In addition, 2-HG is the only product of the transformation of the α-KG by mutated IDH [13]. Interestingly, the amount of 2-HG produced by tumor cells negatively correlates with the prognosis of the disease [14].

2HG behaves as a competitive inhibitor for many enzymes using KG as a cofactor, such as prolyl hydroxylases, histone demethylases, JmjC or 5-methyl hydroxylase of the TET family.

Thereby, the production of 2HG impacts the methylation level of DNA and histones resulting in a hypermethylated DNA, responsible for altering the epigenetic regulation of gene expression [15]. This phenomenon contributes to the differentiation blockage in leukemic cells and thus to the initiation and progression of AML [16]. On the other hand, alterations of prolyl hydroxylase function increase the expression levels of the HIF factor promoting the proliferation of leukemic cells under hypoxic conditions. Concurrently to *IDH* mutations, glutathione concentration is reduced, secondary to the increased NADPH consumption, decreasing the anti-oxidant capacity of cells [17].

Deregulation of Glucose Metabolism

Very intense glucose consumption and very high absorption of this metabolite into the bone marrow have been widely described in AML. AML bone marrow cells display high levels of glucose uptake and high levels of aerobic glycolysis at diagnosis that are correlated with survival and response to chemotherapy. NMR analysis of 443 AML patients showed a specific signature of glucose metabolism, based on the levels of a panel of six metabolites involved in glycolysis (glycerol-3-phosphate, pyruvate, lactate) and in the TCA cycle (citrate, α-KG, 2-HG). This signature confers an adverse prognosis to normal karyotype AMLs, which results in a weak response to aracytin treatment [18]. Similarly, high levels of serum LDH at diagnosis are highly negative prognostic markers in AML [10].

Given the high rate of glucose use by leukemic cells, they can improve their glucose absorption, specifically in case of too low intake levels, by positively regulating the gene *SLC2A5*, which encodes for the trans-membrane glucose transporter, GLUT5. Indeed, *GLUT5* over-expression was associated with a poor prognosis, and conversely, inhibition of this carrier increased the susceptibility of leukemic cells to Ara-C. Similarly, overexpression of mRNA coding for GLUT1, another member of GLUT family, has been associated with poor responsiveness to chemotherapy [19,20].

The PI3K/AKT/mTOR pathway is frequently implicated in deregulation of glycolytic activity. The hexokinase HK2 which catalyzes the first step of glycolysis could be activated by PI3K/AKT dependent mechanisms. mTOR plays an important role in leukemic cell addiction to glucose, through regulation of G6PD (Glucose-6-Phosphate Dehydrogenase), which is an important source of NADPH and which intervenes in the maintenance of the energy metabolism of leukemic cells. In addition, overexpression of G6PD is strongly correlated with an adverse prognosis in AML patients [21]. Indeed, inhibition of G6PD by nicotinamide induces AML blast apoptosis while non affecting normal hematopoiesis. mTORC1 plays also an important regulatory role in the pentose phosphate pathway that is an important pathway for cell survival in AML and all cancers. MAP kinase, a serine/threonine kinase, also promotes the use of glucose and glycolysis by leukemic cells. Its deletion leads to inhibition of leukemic stem cells by decreasing glucose flow and oxidative stress in the hypoglycemic medullary microenvironment [22] through reducing the expression of GLUT1.

Finally, it has been shown that leukemic cells can induce complex variations of the metabolic homeostasis of the host, in order to ensure high glucose concentrations in the bone marrow. In particular, leukemic cells cause increase of insulin resistance and inhibition of insulin secretion by tissues [23]. Such modifications result from an increased production of IGFBP1 by adipose tissue.

Deregulations of Glutamine Metabolism

Glutamine, which is *either produced in* the cell or imported by the glutamine transporter SLC1A5, plays a particular role in normal cells. It fuels the TCA cycle, through regulation of glutathione production and impacts on mTORC1 activity, via regulation of leucine export [24].

Glutamine is particularly involved in the metabolic reprogramming of cancer and leukemic cells [25]. Leukemic cells use glutamine as a source of carbon for energy production

via the TCA cycle. Therefore, leukemic cells have developed an addiction to glutamine [26] on which they depend for survival and proliferation. Consequently, plasma glutamine concentrations in AML patients are much lower than those found in healthy individuals and that KO of *SLC1A5* leads to apoptosis of AML cell lines. Similarly, glutaminase (GLS) is found to be strongly expressed in AML, making it a critical factor for TCA cycle activity [27].

Indeed, many studies have shown that targeting glutaminolysis in AML is a good strategy in the search for new therapeutic agents. This is particularly the case in mutated *FLT3-ITD* AML, which are highly dependent on glutamine to produce energy [28,29]. Targeting glutamine absorption leads to efficient anti-leukemia responses, which may be related to the impact of Glutamine levels on mTORC1 activity [30]. The balance between glutaminolysis and TCA cycle activity therefore appears to be essential in the survival of leukemic cells [10,31]. Moreover, it has been shown that L-Asparaginase which could catalyze the degradation of glutamine suppresses mTORC1 activity leading to AML cell apoptosis. Thereby, this agent well known for its antileukemic activity in ALL, has been proposed for the treatment of AML and recently, an impact on refractory AML has been reported.

Finally, glutamine plays an essential role to control redox homeostasis in AML cells, through its impact on glutathione synthesis and on oxidative phosphorylation in the mitochondria. Blockade of glutathione production through a specific inhibitor provokes accumulation of mitochondrial ROS in multiple AML types and subsequently apoptotic cell death.

Deregulations of Lipid Metabolism

Lipids play a particularly important role in energy production as well as the synthesis of signaling macromolecules for cells. The lipid balance depends on the balance between the lipid intakes and their consumption by oxidation (fatty acid oxidation-FAO) which feed the TCA. As a result, the absorption and consumption of fatty acid (FA) impacts important aspects of the

biology of cancer cells [32]. Indeed, overexpression of FASN (Fatty Acid Synthase), which is a key metabolic enzyme for the final stage of FA synthesis, provides a growth and survival benefit for cancer cells. Interestingly, FASN expression is affected by certain components that play a critical role in cancer metabolism such as PI3K/AKT/mTOR and MAPK signaling pathways [33,34]. Moreover, under metabolic stress conditions, in addition to the increase in lipogenesis, tumor cells can acquire FA *via* lipolysis to support their growth. They will also tend to recover extracellular lipids to maintain their proliferation and survival [35,36]. This lipolysis is mediated by a key enzyme: Lipoprotein Lipase (LPL).Its high activity has been described in lung cancers. LPL has been also identified as a marker of poor prognosis in chronic lymphoid leukemias [37].

In AMLs, absorption and consumption of FA influence the fate of Leukemic Stem Cells (LSC), their adaptation to the microenvironment and their resistance to drugs [10]. The consumption of FA is increased in leukemic cells in order to meet their needs of lipid biosynthesis [38-40]. In promyelocytic AML, the division of HSCs is regulated by FAO and FAO lowers the threshold for apoptosis and promotes the quiescence of leukemic cells. Several lipid profiles representative of different types of AML have recently been identified by mass spectrometry. Significant differences in the modulation of ceramides and sphingolipid synthesis were found in patients with t(8;21) compared to those with inv(16) or normal karyotype [41]. Also, *Stuani et al.* highlighted deregulations of lipid metabolism in a IDH1 mutated cell model of AML, compared to the un-mutated model. These differences are mainly characterized by an increase in phosphatidylinositol, sphingolipids, free cholesterol and monounsaturated FA in mutated IDH cells [42]. This, once again, highlights the impact of cytogenetic characteristics and mutational status of AML patients on cellular metabolism. Finally, a recent study showed a decrease in total FA in plasma of AML patients as well as a reduction in phosphocholine, triglycerides and plasma ester cholesterol. Conversely, arachidonic acid appears to be increased in AML plasma, suggesting its involvement in the cancer phenotype [43].

The critical role of FA metabolism in AML is underlined by its impact on patient prognosis and the subsequent therapeutic interest of targeting this metabolism. A recent study showed that patients with rapidly progressing AML and who did not respond to treatment, had alterations in lipid metabolism compared to other patients [39]. ATP Citrate Lyase (ACL), the enzyme that catalyzes the production of acetyl-coA causes a blockade in cell growth when inhibited in leukemic cell models [44] and has also been shown to be associated with a favorable prognosis in AML patient with low levels of ACL.

In this context, several teams have tested different molecules to inhibit FAO. Among them, etomoxir sensitizes leukemic cells to apoptosis and decreases the number of quiescent progenitors [45]. Also, the molecule Avocatin B combined with a standard chemotherapy agent (AraC) induces apoptosis of AML cells and inhibition of cell growth [46]. Finally, *Picou et al* tested the impact of polyunsaturated FA on AML cell lines and on patient blasts [47]. This type of FA inhibited mitochondrial oxidative phosphorylation and increased glycolysis and oxidative stress, resulting in death of cancer cells. On the other hand, CPT1A, a sub-unit of the mitochondrial enzyme on which FAO relies, was found overexpressed in bone marrow samples from AML patients compared to normal bone marrow. This overexpression was strongly associated with an unfavorable prognosis [48]. FAO could therefore contribute to a mechanism promoting resistance to therapies. Indeed, resistant AML cells showed overexpression of the FA transporter, CD36, and a very intense FAO [49,50]. FAO-derived NADPH can be a decisive electron donor for leukemic cells under therapy, which then allows them to combat oxidative stress, and can be used in the anabolic processes necessary for cell division [10,51]. In addition, a new element, PHD3 (Prolyl Hydroxylase Domain 3), has been discovered as potentially interesting in the metabolism of AML. It is part of a class of enzymes capable of coordinating metabolism in response to changing cellular conditions. The normal function of this KG-dependent enzyme is to suppress FAO activity under conditions of nutrient abundance in the cellular environment. However, it has been shown that in AML, this enzyme is present at a low level, which leads to the

persistence of FAO regardless of external nutrient conditions. This could make PHD3 a very good biomarker of AML [52].

Deregulation of Phospholipid Metabolism

Very little information is available regarding the potential links between the deregulation of phospholipid metabolism and leukemogenesis. A team recently studied the plasma phospholipid profile of AML patients, and showed a decrease in free total FA, including phosphocholine, which is likely due to increase FA oxidation in AML cells [39]. This would have an adverse prognosis. Moreover, Wang *et al* showed lower phosphocholine (PC) levels in sera of AML patients, probably linked to the excessive need for this metabolite for leukemic proliferating cells. Interestingly, they also observed that this metabolite was expressed at a higher level in the serum of intermediate prognosis patients compared to favorable prognosis ones. This argues in favor of an adverse prognosis of the PC [39]. On the other hand, a significant decrease in phosphatidylserine (PS) and sphingomyelin (SM) have been described in the blood mononuclear cells of AML patients compared to healthy donors [53].

Finally, a much more recent study highlighted the involvement of an enzyme, the Taffarazine (TAZ) not yet described in AML [54,55]. TAZ is a mitochondrial enzyme that catalyzes the maturation of cardiolipin, and is therefore a key enzyme for phospholipid biosynthesis. TAZ suppression leads to a decrease in cancerous cell proliferation, inhibition of clonogenic growth and induction of leukemic cell differentiation, while preserving normal hematopoiesis. Interestingly, the study of the phospholipid profile of AML cells when TAZ is suppressed shows that, in addition to an expected decrease in cardiolipin, a decrease in phosphatidyl-ethanolamine and an increase in phosphatidylserine. Targeting phospholipid metabolism therefore appears to be a promising hypothesis for targeting LSC [54,55].

II-The Particular Role ok LKB1 in Pathogenesis of AML

One of the major controllers of adequacy between the energy production level and proliferation signals is the mTOR kinase (target of rapamycine). mTOR integrates mitogenic signals from PI3K/AKT that are based on the nutrient or energy reserves enabling synthesis of proteins, amino acids and lipids necessary for cell proliferation. Effectors modulate mTOR activation state via phosphorylation of the TSC1-TSC2 complex [56,57].

PI3K/AKT/mTOR pathway is one of the most frequently altered in tumors and in particular in AML [58], by mutation or due to deregulation of tumor suppressor genes such as *PTEN* [59]. These deregulations induce signals of proliferation and cell growth, but also have a significant impact on metabolic activity. AKT can induce an increase in glycolysis through increased glucose captation, FOXO inhibition or activation of protein glycosylation enzymes [60]. In addition, AKT strongly stimulates mTOR activity by inhibiting its degradation, thus promoting protein and lipid synthesis even in energy deficiency conditions. Similarly, the p53 protein, whose gene is frequently mutated or inhibited in tumors, is an important regulator of metabolism highlighting the close links between proliferation and metabolic changes in tumor cells [61].

One of the major signals detected by mTOR is the level of ATP (thus the energy level of the cell). When the intra-cellular ATP concentration decreases, the AMP/ATP ratio is modified resulting in the recruitment of AMPK that will inhibit mTOR signaling [62]. The phosphorylation of AMPK is performed by LKB1 which is a serine threonine kinase encoded by the STK11 gene located on chromosome 19p13.3 in humans [63,64]. This enzyme is involved in the regulation of cellular metabolism, while also governing important processes such as cell proliferation, polarity and migration, all of which being crucial in carcinogenesis [65]. Previous studies have identified several effectors regulated by LKB1. Some of them include major proteins in carcinogenesis, implicated in several cancers such as p53 [66] and the tumor suppressor gene PTEN (Phosphatase and

TENsin homolog) [67]. However, one of the most important targets of LKB1 is the AMP-dependent kinase protein (AMPK) activated by phosphorylation of threonine 172 [68], demonstrating the major role of the LKB1/AMPK axis in detecting cell energy levels. It has also been discovered that 14 other AMPK-like kinases are activated by LKB1, but of these, only AMPK 1 and 2 (catalytic components of AMPK) appear to be activated under low-nutrient conditions [69].

LKB1 was identified as a tumor suppressor when it was discovered that germ mutations occurring in the gene encoding for *LKB1* were responsible for a rare disease called Peutz-Jeghers syndrome (SPJ), which causes a predisposition to cancer [70]. In addition, LKB1 mutations or 19p chromosome losses are also implicated in sporadic pulmonary adenocarcinomas and somatic mutations of *LKB1* have been shown to synergize with human papillomavirus infections and promote disease progression in approximately 20% of cervical cancer patients [71].

Recent studies have shown that LKB1 may play a role in the pathophysiology of several hematological cancers, including AML [72]. However, in AML, unlike to what is observed in many solid tumors, the tumor suppressor role of LKB1 is controversial. LKB1 appears strongly expressed in leukemic stem cell population. Inhibition of LKB1 activity in these cells appears to stop their proliferation and make them more sensitive to chemotherapy. By studying gene expression profiles in a leukemic cell line (KG1a) expressing LKB1, it was found that the MAPK pathway was activated by LKB1 but independently of AMPK [73]. Therefore, unlike its tumor suppressor role in most cancers, in AML LKB1, via mechanisms independent of AMPK can act as a tumor promoting factor, even if this mechanism remains to be confirmed. Conversely, the LKB1 pathway through AMPK-dependent mTOR inhibition, is responsible for inhibiting protein translation and thus cell proliferation [72], in favor of a tumor suppressor action. In addition, in 7% of a cohort of 54 patients, a polymorphism in LKB1 was found in position 354 causing an amino acid change from leucine to phenylalanine (Leu354Phe) in the C-terminal

protein domain. This polymorphism would cause a decrease in AMPK activation mediated by LKB1 [74] and could have an unfavorable prognostic value in AML.

Another set of effector kinases activated by LKB1 are the SIK2 and 3 (Salt-Inducible Kinase) [75]. This LKB1-SIK2/3 axis has been very recently involved in the progression of AML. The activation of SIK2 and SIK3 following LKB1-mediated phosphorylation, promotes activation of the histone deacetylases (HDACs), which include HDAC 4, 5, 7 and 9 [73] Of these, HDAC4 is specifically involved in the progression of AML. Normally, when HDAC4 is active, it inhibits a repressive factor called MEF2C (Myocyte Enhancer Factor 2C), which has been found to be highly overexpressed in leukemias [72,74]. SIK2 and 3 by phosphorylating HDAC4 inhibit its deacetylation activity and therefore, HDAC4 cannot inhibit MEF2C leading to de-repression and expression of genes that promote the proliferation of AML cells. Thus, seen from this side, LKB1 seems to be pro-tumoral with regard to AML The treatment of these leukemic cells with SIK2/3 inhibitors confers sensitivity to drugs and death of these cells, giving rise to a potential therapeutic value for SIK inhibitors in AML cases positive with an activation of the LKB1-SIK axis [74].

III-Deregulation of Redox Balance

Reactive oxygen species (ROS) are the products of molecular oxygen metabolism by cells. They include superoxide anion O_2^- hydrogen peroxide H_2O_2, hydroxyl radicals and NO nitrogen monoxide. They can be generated by the electron transport system in the mitochondria and by the NADPH Oxydase (NOX) complexes at the membrane level [76]. Within the hematopoietic niche, hematopoietic stem cells (HSCs) proliferate in the most hypoxic part of the bone marrow and their differentiation roughly follows the oxygen gradient (from 6 to 1%) present in this hematopoietic niche [77]. The redox balance which reflects the balance between oxidizing and anti-oxidant (reducing) species, acts as a specific effector of certain regulatory effectors, and therefore directly impacts the cellular fate of the HSC. Hypoxia in the hematopoietic niche regulates NOX by keeping

ROS levels low, which helps to keep HSCs in quiescence [78], while protecting them from DNA damage, which can be induced by ROS [79]. Conversely, an increase in ROS can lead to changes in the phenotype of HSCs and promote their differentiation to progenitors [80]. Indeed, reactive oxygen species levels regulate transcription factors such as FOXO3, GATA-1, NFE2 and therefore play a decisive role in the engagement or progress of the maturation and differentiation processes of hematopoietic stem cells [81].

Redox-Dependent Changes in Leukemogenesis and AML

Excessive production of Reactive Oxygen Species has long been described in malignant hemopathies and in particular in acute myeloid leukemia (AML) or chronic myeloid leukemia (CML) [82]. Oxygenated radical levels are higher in AML blasts compared to normal leukocytes [83].

However, the mechanisms between ROS production and leukemia progression are still incompletely understood. Disturbances in anti-oxidant activities, particularly those dependent on FOXO transcription factors, can lead to an environment conducive to the generation of tumor clones. An excess of ROS production via the activation of NOX2 [84] has been shown in CD34 cells expressing the *RAS* mutation. The same is true with the *FLT3-ITD* and *NOX4* mutation [85]. Among other possibilities, inactivation of PTEN phosphatase by H_2O_2 may promote activation of the AKT pathway observed in AMLs.

AKT and *FLT3* are consistently activated in the majority of AML, suggesting inhibition of FOXO function and an increase in ROS levels compared to normal HSC [86], as well as regulation of leukemic cell survival, associated with resistance to chemotherapy [87]. Indeed, it appears that the aggressiveness and poor prognosis associated with *FLT3-ITD* mutated AML is due to the increase in endogenous ROS, which is produced by STAT5 transcription factor signaling and the activation of RAC1, which is an essential component of ROS-producing NOX

[88]. The expression of the *RUNX1-RUNX1T1* protein, associated with t(8;21), leads to a ROS-dependent increase in the survival and proliferation of hematopoietic precursors [78]. Finally, *IDH1/2* mutations are accompanied by a reduction in the synthesis of glutathione, secondary to the decline in NADPH production, which could increase the deleterious effect of ROS induced by the overproduction of 2HG [16]. Thus, significantly pro-oxidant conditions generally lead to cell death, but genetic changes in leukemic clones allow for survival and proliferation. Blast development could therefore be the result of a deficiency in the detection, response and/or integration of signals induced by partial oxygen pressure and redox potential.

Conversely, ROS-inducing chemotherapy selectively eradicates leukemia stem cells. Thus, inter-individual variations (SNP) of the gene coding for NADPH oxidase, the key enzyme in ROS emission, appear to influence the effectiveness of chemotherapy in AML [89]. In that meaning, a correlation between oxidative stress and the incidence of AML relapses was found as well as the therapeutic value of molecules targeting iron homeostasis and ROS production in chemotherapy-resistant AMLs [90]. Finally, our team recently showed that AML patients had a deregulated redox balance related to their molecular status, involving leukemia cells, non-tumor cells and the antioxidant system, which play a major role in the prognosis of patients [91]. In particular we showed that depending on the capacity of mitochondria to mobilize their spare capacity, we can identify different prognosis sub groups and that both high reduced/oxydative glutathione ratio and high thiol levels at diagnosis were associated with a lower risk of death.

The other major source of ROS is mitochondria. This production participates in redox signaling in normal cells but can also promote the tumor process. Although most tumor cells switch their metabolism to aerobic glycolysis (Warburg effect), mitochondria appear to remain functional and above all capable of producing ROS during the tumor process. In particular, in hypoxic conditions that normally decrease the production of mitochondrial ROS, leukemic cells can increase this production of ROS [92]. This increase promotes the proliferation of tumor

cells in a hypoxic environment, and the progression of the disease by their potential to damage DNA.

Mitochondrial ROS can also promote the tumor process by inducing damage to nuclear or mitochondrial DNA [93]. In addition, mutations in mitochondrial DNA cause a deficit in the activity of the mitochondrial respiratory chain and are associated with the overproduction of ROS [94]. Numerous studies have observed an increase in mitochondrial ROS induced by anti-leukemic treatments during apoptosis [95] but few studies have studied the pathophysiological role of mitochondrial ROS in leukemia. Mitochondrial ROS emitted by CD34 blasts appear to be weaker than those produced by normal CD34s [82]. However, a recent study also showed that blasts showed increased sensitivity to mitochondrial oxidative stress. These blast cells were characterized by an increase in mitochondrial mass, but without a concomitant increase in their respiratory activity [96].

Indeed, mitochondria play a key role in metabolism as well as in many processes such as apoptosis, calcium homeostasis and more recently in phospholipid metabolism (see above). Thus, dysfunctional mitochondria are highly involved in carcinogenesis and directly or indirectly in tumor cell replication, insensitivity to antiproliferative signals, sustained angiogenesis, invasiveness, avoidance of immune response [97,98]. Leukemic cells are characterized by an increase in mitochondrial mass, but without a concomitant increase in respiratory activity [96,99]. Although some tumor cells switch their metabolism to aerobic glycolysis (Warburg effect) most AML use oxidative phosphorylation to meet their energy needs. The microenvironment in particular stromal cells plays a role as a donor of mitochondria through the creation of nanotubes [100,101]. Thus, chemotherapy resistant leukemic cells have high levels of ROS and a high mitochondrial mass, which seems to be related to the transfer of mitochondria from the bone marrow to the blasts, which requires the activity of NOX2 that stimulates the release of mitochondria by stromal cells [100]. Aracytin-resistant leukemic cells show high use of oxidative phosphorylation, increased β-oxidation and hyperexpression of CD36 [51]. Therefore, targeting the synthesis of mitochondrial

proteins or the oxidation of fatty acids appears to be a promising way to improve therapeutic efficiency.

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