Book Chapter

Amino Acid Metabolism in Acute Myeloid Leukemia

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Abstract

The role of metabolic alterations in cancer cells has been discussed and clarified over the years. Researchers focused on key metabolites and the mechanisms they initiate in cancer. Acute myeloid leukemia (AML) is one such cancer that is dependent on certain metabolites and the pathways they trigger.

Even though several advances have been made in understanding the mechanisms underlying the initiation and progression of AML, therapies targeted toward these mechanisms, although potent, do not always achieve the desired effect. Hence, studying the metabolic alterations in AML is one of the many approaches researchers currently employ in order to glean a deeper understanding of the intricacies that govern this cancer. Amino acids are crucial players in AML. They trigger several cell survival and replication processes, as well as modulate key epigenetic processes – all of which are critical in carcinogenesis. Moreover, several amino acids have been found to play a role in the maintenance of leukemic stem cells, which are correlated to poor prognosis in AML. The role of a few amino acids in AML are highlighted in this review.

Introduction

Almost a century ago, Otto Warburg proposed the first metabolism-related alteration in cancer when he showed that cancer cells selectively prefer aerobic glycolysis for their propagation [1]. This led to a revolutionary development in the field of metabolic reprogramming in cancers. Metabolic pathways involved in carbohydrate, fatty acid and amino acid metabolism have been explored. More specifically, glycolysis and the tricarboxylic acid cycle (TCA) and their alterations in cancer have been largely studied. Much attention was also focused on amino acids and their role in cancer metabolism [2]. All these studies established that tumors differ metabolically from normal counterpart tissues [3]. Glucose and amino acids are important substrates used by cancer cells. Glutamine has been deemed a "super nutrient" as it participates to various signalling pathways including the TCA via anaplerosis and also contributes to redox homeostasis, a critical regulator of proliferation in cancer [4]. Moreover, in 2016 a study revealed that other amino acids which are consumed at slower rates than glutamine and glucose are major fuels for proliferating mammalian cells [5]. This is a major incentive to further investigate the role of amino acids in cancer cell metabolism. Acute myeloid leukemia (AML) is a highly heterogeneous bone marrow cancer characterized by uncontrolled proliferation of

immature myeloblasts (referred to as 'AML blasts') in the bone marrow and peripheral blood, causing failure in erythropoiesis [6]. This leukemia is most common in adults about 65 years of age and fairly uncommon in people less than 45 years, and has an overall five years survival rate after diagnosis of 28.7% [6,7]. Like most cancers, the role of metabolic reprogramming has been established in AML [8]. In this review, we discuss the role of certain amino acids, mostly non-essential ones, in cancer and more specifically in AML.

Synthesis of Amino Acids and its Link to Other Metabolic Pathways

A total of 20 amino acids are the known building blocks for proteins and are categorized into two major groups on the basis of dietary requirements as essential amino acids (EAAs) and non-essential amino acids (NEAAs) [9]. Of these 20 amino acids, 9 are thought to be essential and include Valine, Leucine, Isoleucine, Histidine, Methionine, Tryptophan, Phenylalanine, Lysine and Threonine. The remaining 11 are non-essential and consists of Glutamic acid, Glutamine, Proline, Glycine, Tyrosine, Alanine, Serine, Arginine, Asparagine, Cysteine and Aspartic acid [10]. As metabolism is a highly dynamic process, amino acid metabolism is interconnected with several other cellular pathways. Amino acids are building blocks of proteins. Furthermore they are degraded to provide energy or consume energy for their own biosynthesis.The metabolic fate of various amino acids is called anaplerosis or cataplerosis of amino acids, which represents the replenishing or removal of TCA cycle intermediates, respectively [11]. Glucogenic amino acids are those that are ultimately channelled into gluconeogenesis via intermediate conversions into TCA cycle products oxaloacetate, pyruvate, succinyl-CoA, fumarate or α-ketoglutarate (α-KG); while ketogenic amino acids form ketone bodies through intermediate conversions into acetyl-CoA [12]. The glucogenic amino acids include alanine, glycine, serine, cysteine, asparagine, aspartic acid, proline, glutamine, glutamic acid, arginine, methionine, valine and histidine; while the ketogenic amino acids comprise of leucine and lysine. The remaining five which include threonine, tryptophan, tyrosine, phenylalanine and

isoleucine are both – glucogenic and ketogenic. [11]. Overall, the cataplerotic and anaplerotic metabolism of amino depends on the metabolic and nutritional condition of the body [11]. The above mentioned reactions utilize the carbon skeleton of amino acids, however, the nitrogen of amino acids is removed first through various transamination reactions, all using the coenzyme pyridoxal phosphate. This nitrogen is ultimately funnelled into the urea cycle for its removal from the body [13]. Thus, the breakdown and utilisation of amino acids comprises a number of enzymes and forms a highly dynamic and intricate metabolic network.

Role of Amino Acids in Cancer Manifestation

A variety of processes that suggest amino acids to be crucial players in cancer progression and disease manifestation have been discovered. One widely accepted theory is that as cancer cells proliferate profusely, they have a higher demand of amino acids. Cancer cells glean the required amino acids by mechanisms like protein scavenging, but can also depend on their environment for a supply of NEAAs [14]. Another mechanism for cancer cells to meet the high requirement of amino acids is to increase the number of amino acid transporters on the cell membrane [15]. For example, the SLC7A5 transporter, belonging to the System Leucine-preferring amino acid transporters, also known as L-amino acid transporter 1 (LAT1)is overexpressed on the metastatic lesions of various cancers as compared to their primary site of origin [16]. Another transporter of neutral amino acids called ASCT2 (or SLC1A5) has been specifically implicated in AML [17]. ASCT2 mediates transport of Glutamine, Alanine, Cysteine, Serine, Valine and Threonine [18,19]. The constitutive deletion and thus loss of ASCT2 function has been shown to disrupt the influx of leucine (that is dependent on glutamine; discussed further under glutamine metabolism) is responsible for mammalian target of rapamycin (mTOR) signalling and induces apoptotic cell death in AML cells [17]. Moreover, factors controlling oncogenesis like the suppressive microRNAs miR-126 and the activation of the c-myc gene (an important regulator of cell proliferation) are also responsible for the downstream expression of LAT1 [20,21].

This suggests that amino acid uptake and metabolism are crucial mechanisms in carcinogenesis.

Glutamine and its Metabolism in Cancers and AML

According to a study published by Kroemer et al in 2008, glucose and glutamine are two necessary ingredients for the uncontrolled proliferation and propagation of cancer [22]. Glutamine is abundantly present in human plasma (0.6 – 0.9 mM) as well as inside cells (~20 mM). These concentrations are beneficial to cell physiology and to carcinogenesis [23]. Glutamine contributes to a number of important processes like generation of NEAAs , DNA replication via purine and pyrimidine biosynthesis, metabolite generation to sustain mitochondrial metabolism, synthesis of fatty acids for cell proliferation, redox homeostasis by supplying antioxidants to eliminate reactive oxygen species (ROS)-induced damages and activation of various pro-tumoral signalling mechanisms. Glutamine is so crucial in cancer cell metabolism that the process of anaplerosis by which glutamine provides TCA cycle metabolites and upholds the metabolic prowess of cancer cells has been called another "hallmark of cancer metabolism" [24-26]. In acute myeloid leukemia the bidirectional amino acid transporter SLC1A5 takes up leucine while simultaneously exporting glutamine. Leucine activates mammalian target of rapamycin complex 1 (mTORC1) stimulating protein synthesis, thus sustaining AML [27,28]. Hence, removal of glutamine supply to these AML cells leads to the inhibition of mTORC1 and subsequent hindrance in protein synthesis, and ultimately death of AML cells via apoptosis [29]. The enzyme glutaminase is encoded by two different genes: GLS1 which produces two splice variants – the kidney-type glutaminase and glutaminase C; and GLS2 which encodes the liver-type glutaminase [30]. Of these two genes, the products encoded by GLS1 are highly expressed in cancer, including AML [28,31]. Proper functioning of glutaminase, that causes glutamine deamination to glutamate, which is then converted to α-KG to enter the TCA cycle, has been found to be elevated and essential to various cancers [32,33]. Hence, using a glutaminase inhibitor CB-839 led to an instable level of ROS in the mitochondria of AML cells due to

impaired generation of the antioxidant glutathione (GSH), which culminated in the mitochondrial apoptotic death of these AML cells [34]. As the mitochondrial apoptotic pathway is induced, glutaminase inhibitor treatment in combination with BCL-2 inhibitors confers a synergistic effect in eliminating AML blasts [35]. Moreover, in AML cases with isocitrate dehydrogenase (IDH) mutations, use of this CB-839 GLS inhibitor also led to a decrease in the production of 2-hydroxyglutarate, an onco-metabolite. This further emphasizes the importance of glutamine metabolism in AML [31,36].

Targeting the Linked Asparagine and Glutamine Metabolisms in AML

Asparagine is one of the eleven NEAAs and hence, is produced by the body itself in a reaction catalysed by the enzyme asparagine synthetase (ASNS), which uses aspartate and glutamine as substrates to produce asparagine and glutamine in a transamination reaction utilizing ATP [9,37]. However, if cells are unable to produce asparagine by this mechanism, they can be targeted and killed upon treatment with L-asparaginase (ASNase), an enzyme that hydrolyses glutamine and asparagine resulting in their subsequent depletion in the bone marrow and peripheral blood [23,38,39]. Such treatment with ASNase has been potent in haematological malignancies like lymphomas and acute lymbhoblastic leukemia (ALL), as the cancer cells in these cases lack ASNS and hence cannot replenish the lost asparagine post ASNase administration [40,41]. These low levels of asparagine and glutamine ultimately trigger certain downstream mechanisms like – (a.) activation of GCN2, a kinase that phosphorylates eIF2α, which causes a decrease in cellular protein synthesis [42]; (b.) induction of apoptosis to deplete lymphoma cells [43]; (c.) inhibition of mTOR – an effect specific to the glutamine-depleting function of ASNase, which also leads to a decrease in protein synthesis and disrupts cell survival mechanisms [29,44]. Hence, glutamine reduction is a crucial process that accompanies the anti-cancer effects of ASNase [23]. As compared to ALL, AML blasts are heterogeneous for ASNS expression and are generally more responsive to glutamine deprivation [38]. Heterogeneity could

contribute to AML resistance to asparaginase-based treatments. Interestingly, a study published in 2019 suggested that the $CD34^{+}CD38^{+/-}$ leukemic stem cells (LSCs) from AML patients show an intrinsic sensitivity to L-asparaginase treatment, but the microenvironment in the bone marrow has monocytic cells that can produce a lysosomal protease called cathepsin B, that is capable of degrading the ASNase. The AML cells (belonging to the FAB M5 category in this case) themselves may also produce this protease to inactivate ASNase and reduce treatment efficacy [45]. Moreover, ASNase with intrinsic glutaminase activity has been shown to be more effective in treating AML. One such ASNase, called Erwinase (derived from *Erwinia chrysanthemi*), has a glutaminase activity that is ten times superior to that of *E. coli* [46]. Of note, combination of ASNase with cytarabine in AML therapy led to an improved survival length, the administration sequence of the two drugs being critically important [47,48].

Targeting Arginine Metabolism in AML

Arginine has been deemed "semi-essential" as under normal physiological conditions, the body is capable of generating arginine from other amino acids like proline, glutamine and glutamate [49]. However, in pathologic conditions like cancer, cells become auxotrophic for arginine [50]. Cancer cells rely on arginine as it generates polyamines, components of chromatin necessary for cell growth, proliferation and metastasis [51,52]. Arginine is also important for the generation of nitric oxide (NO), low levels of which seem to be pro-tumoral whereas high NO levels lead to cell death [53-55]. The enzyme argininosuccinate synthetase 1 (ASS1) of the urea cycle uses aspartate, citrulline and ATP to form argininosuccinate, which is converted to arginine by another enzyme called argininosuccinate lyase (ASL) [56]. Immunohistochemical analysis of AML samples confirmed that most cases did not stain positively for ASS1, while expressing ASL normally – making these AML cells dependent on extracellular sources of arginine [57,58]. Further analysis revealed that methylation of the ASS1 gene promoter in AML samples as well as other lymphoma samples was the cause for arginine dependence in these cancers

[57,59]. Moreover, it was found that cancer cells deficient in arginine undergo apoptosis or autophagy, or both when deprived of arginine [60]. It was shown that a pegylated form of arginine deiminase called ADI-PEG, derived from *Mycoplasma,* converts arginine to citrulline, thereby depleting AML cells from arginine to. AML cells lacking ASS1 enzyme showed activation of caspases, whilst cells with functional ASS1 were resistant to ADI-PEG 20 induced arginine deprivation. The same investigations showed that in patients deficient for ASS1, combination therapy using cytarabine and ADI-PEG 20 proved to be more potent than either single treatment [58]. Another enzyme arginase (ARGase) also participates in the urea cycle by converting arginine to ornithine [56]. In 2015, researchers showed that along with ASS1, another enzyme of arginine metabolism – ornithine transcarbamylase (OTC) was also deficient in AML blasts, with the simultaneous upregulation of cationic amino acid transporters CAT-1 and CAT-2B, which helped in the uptake of arginine from the extracellular milieu to make up for loss of functional ASS1 and OTC. They formulated the pegylated recombinant human arginase called BCT-100, which like ADI-PEG 20 depleted the arginine supply from AML blasts along with the intracellular arginine reserves, but unlike ADI-PEG 20 did not treat only ASS1-deficient AML, but both – ASS1 and OTC-lacking AML. BCT-100 also showed synergy with cytarabine treatment in AML [61]. This lead to development of "arginine deprivation therapy" in cancers like AML, wherein the enzymes ASS1 and OTC served as biomarkers for response or resistance to such treatment.

Alanine Metabolism in Cancers

The production of alanine in the cell is regulated by the enzyme alanine aminotransferase (ALT), also known as glutamate-pyruvate transaminase (GPT) – which uses glutamate and pyruvate to generate alanine and α-KG in a reversible reaction [62]. Two isoforms of ALT encoded by two different genes on two chromosomes exist – ALT1 (or GPT1) is encoded from chromosome 8q24.3 and represents the cytosolic protein, whilst ALT2 (or GPT2) is encoded from chromosome 16q11.2 and generates the mitochondrial protein [63]. The role of ALT has

been described in lung carcinoma cells. ALT inhibition using cycloserine and chloroalanine was found to disrupt alanine production and glucose intake by these cells, followed by a subsequent activation of mitochondrial metabolism - all of which ultimately led to a decline in proliferation and acquisition of a malignant potential [64]. Moreover, ALT function appeared to play a role in the development of extracellular matrix (ECM) through the formation of α-KG, which causes collagen hydroxylation by increasing certain enzymatic activities and thus acting as a crucial contributor to metastatic breast cancer [65]. Another instance of alanine metabolism has been described in pancreatic ductal adenocarcinoma (PDAC). In this case, the pancreatic stellate cells (PSCs) in the stroma around the tumor secrete alanine (via an autophagic mechanism induced by the tumor), which is then taken up by the PDAC tumor itself and fuelled into the TCA cycle via anaplerotic reactions that generate pyruvate. It is interesting to note that this secreted alanine is the major carbon source for the TCA cycle in these tumors, even beating the carbon derived from glutamine and glucose, which are the two main cancer promoting and cell survival carbon providers (66). This allows the use of glucose and glutamine for other reactions, depending on the demand of the cancerous tissue [66,67]. When it comes to AML, no direct link between alanine metabolism and cancer progression has been described yet. However, the liver kinase B1 (LKB1) is an important “master kinase” of metabolism in various cancers, and is thought, for the most part, to act as a tumor suppressor in AML by downregulating the mTOR pathway and subsequently decreasing cell growth and survival [68]. Recently, it was found that LKB1 regulates ALT activity and thus the pyruvate-alanine conversion in neural crest cells, in a pathway that relies on mTOR [69]. As LKB1 is highly active under nutrient-deficient conditions in AML, it could potentially affect alanine metabolism by regulating ALT activity in a similar manner, although this premise remains hypothetical.

Role of Amino Acids in "Stemness" and Leukemia Progression

It has been established that for hematopoietic stem cell (HSC) sustenance, valine is essential, and depriving these HSCs of valine supplementation leads to a decrease in their overall numbers. More specifically, valine is necessary for the self-renewal of HSCs [70]. Valine is a branched-chain amino acid (BCAA) – along with leucine and isoleucine [71]. BCAAs are involved in nutrient sensing (especially via leucine) and play a role in modulating the overall metabolism of cells, cell proliferation and protein synthesis via the AMPK and mTOR signalling pathways [72,73]. Catabolism of BCAAs takes place in two steps: the first is catalysed by the transaminases BCAT1 (cytosolic) and BCAT2 (mitochondrial), which use a BCAA and transfer nitrogen to α-KG leading to glutamate and the corresponding branched-chain keto acid [74]. The latter and final step is catalysed by a common branched-chain α-ketodehydrogenase (BCKDH) complex, which ultimately generates TCA cycle metabolites succinyl-CoA or acetyl-CoA [75]. This BCKDH has three subunits (E1, E2, E3-ligase) and a phosphatase activity called PPM1K. It dephosphorylates the E3-ligase component and activates BCKDH activity to promote BCAAs breakdown [76]. As BCAAs are crucial to maintain HSCs, PPM1K functioning is important to maintain a certain threshold of these BCAAs and has been found to sustain "stemness" of not only HSCs, but also leukemia initiating cells (LICs) by ubiquitination-mediated regulation of p21 and MEIS1 [77]. Moreover, PPM1K was found to promote AML development, thus highlighting the involvement of BCAAs in AML [77]. The role of BCAAs has now been sufficiently investigated to postulate that AML cells are "addicted" to these amino acids for their stemness and propagation [78]. Leukemic stem cells (LSCs) are known to have a low level of OXPHOS (on which they rely for metabolism, as opposed to glycolysis-dependent metabolism) as compared to other AML blasts, which have a relatively high OXPHOS signature [79]. However, LSCs seem to rely on BCL-2 (anti-apoptotic) and glutathione in order to sustain the low level of their mitochondrial output and avoid cell death [80]. Hence, using venetoclax (a BCL-2 inhibitor) has

been proposed to selectively target LSCs in AML [81]. LSCs harvested from primary AML tissue are enriched in amino acids, which sustain the aforementioned OXPHOS in these LSCs and hence promote their survival [82]. Azacitidine (a hypomethylation drug) in combination with venetoclax has been previously used in clinical trials to treat *de novo* AML and show a relatively strong response by mechanisms which target LSCs [83]. Jones et al used this combination therapy and saw that it lowered the amino acids load in LSCs, which could be another mechanism to decrease leukemogenesis and propagation of AML [82]. Overall, research has identified amino acids to sustain LSCs and hence given incentive to study the role of amino acids in blood cancers like AML.

Amino Acid-Mediated Epigenetic Regulation in AML

The role of metabolic pathways in the regulation of epigenetic processes has been established in recent years [84]. It is known that epigenetic alterations can modify expression of various metabolic genes (like those governing amino acid metabolism) Conversely, many epigenetic enzymes depend on and interact with various metabolites [85]. As discussed above, α-KG is produced through various transamination reactions involving the amino acids glutamate and alanine, and also through the enzymes glutamate dehydrogenase and IDH [63,84]. Epigenetic enzymes TET (ten-eleven translocation) and JHDM (Jumonji-C domain containing histone demethylase) rely on this α-KG and iron (Fe^{2+}) in the nucleus [86]. In AML, the IDH1 and IDH2 mutations lead to production of the onco-metabolite 2-hydroxyglutarate (2-HG), which competitively inhibits enzymes dependent on α-KG like TET2 (a DNA demethylase), leading to global or specific DNA hypermethylation, which ultimately disrupts HSC differentiation and seems to confer an overall pro-leukemic effect [87,88]. EGL-9 family of hypoxia inducible factor 1 (EGLN1) is another DNA demethylase that uses α-KG as a cofactor and targets HIF-1α for proteasomal-mediated degradation [89]. The enzyme BCAT1 which is involved in BCAAs catabolism regulates the intracellular α-KG reservoir, as discussed above. A proteomic analysis of stem and non-stem cell

groups in AML samples showed enrichment of BCAT1 in LSCs [90]. The role of hypoxia-inducible factor 1α (HIF-1α) plays a major role to maintain LSC populations in AML [91]. AML stem cells that highly express BCAT1 have decreased α-KG concentrations, which hinder EGLN1activity and promote leukemia onset via HIF-1α in LSCs. This mimics the pro-leukemic DNA hypermethylation effect observed in IDH mutant AML cells [90,92]. Hence, depletion of BCAT1 leads to an increase in the α-KG levels and thereby, an increase in EGLN1-mediated degradation of HIF-1α, which disrupts the leukemia-initiating ability of AML stem cells [90]. In conclusion, amino acids are important metabolites that affect cancer onset and propagation through various mechanisms, including epigenetic modifications.

Conclusion

A large amount of research and metabolic networking tools have been able to establish the pivotal role of amino acid metabolism in an array of cancers. Amino acids can be either pro-tumoral or tumor-suppressing, depending on the pathways they trigger in cancer cells [2]. The role of glutamine is well established in various cancers, including AML, and glutamine starvation has been a highly debated area in terms of anti-cancer therapy [15,29]. Even though glutamine evidently affects cancer progression and growth, other amino acids discussed in this review have also been shown to be significant in promoting or disrupting carcinogenesis. The role of asparagine has been highlighted in blood cancers, especially ALL, and hence treatment with L-asparaginase was an important milestone in ALL treatment, but was considered as subpar against AML [23]. However, the discovery of a mechanistic link between glutamine and asparagine in AML made it reasonable to use asparaginases with high glutaminase activit [38]. This provided a much needed boost to asparaginase-based anti-AML therapy. Arginine is another amino acid which has been shown to be an important tumor-promoting metabolite to cancer cells, including AML blasts [57]. The finding of intricate mechanisms that are based on enzymes like argininosuccinate lyase (ASL), that normally function in the urea cycle, led to the development of arginine-

metabolism targeting anti-cancer drugs like ADI-PEG 20 [58]. Researchers were also able to increase the efficacy of standard cytarabine therapy by combining it with ADI-PEG 20, which appears as a success story in AML therapy, especially in cases that are 'auxotrophic' for arginine [58]. Furthermore, the role of alanine has been highlighted in cancer [66]. Although there is no solid evidence of its role in promoting AML, it is supposed to act in tandem wilth the liver kinase LKB1 [69]. Leukemic stem cells (LSCs) are an essential area of research that has been discussed over the years with regard to AML [93]. Not surprisingly, the role of BCAAs and others in maintaining these LSC populations and thus being an important mediator in AML manifestation has been another key discovery [77,82]. Lastly, targeting the role of amino acids and the enzymes modulating amino acid metabolism in epigenetic regulation of gene expression in AML is a more recent and novel approach [89]. The importance of amino acids in regulating cancer metabolism in conjunction with several other metabolic pathways is emerging as an important hallmark that has led to the development of various drugs targeting amino acid metabolism, some of which are in clinical trials [2]. Studies highlighting various aspects of amino acids in cancer are an important pillar of metabolomics research that will certainly shape the future of therapeutic action against cancer. Finally, it did not escape the reader's attention that amino acids are major players in the normal landscape of physiology. It follows that anti-cancer interventions will require a high specificity.

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