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Exploiting leucine metabolism for therapeutic benefit in acute Myeloid Leukemia

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Abstract

Acute Myeloid Leukemia (AML) presents significant challenges in treatment, especially for elderly patients. Conventional therapies have limitations, necessitating the exploration of novel strategies. Metabolic reprogramming in cancer cells offers promise in this regard, with growing evidence pointing to dysregulated leucine metabolism in AML. Leucine deprivation has shown potential in inhibiting leukemic cell proliferation, inducing cell cycle arrest, and enhancing differentiation. Combination therapies involving leucine deprivation and chemotherapy have exhibited synergistic effects. The precise molecular mechanisms of leucine deprivation's anti-leukemic effects, particularly its potential impact on the mTOR signaling pathway, require further elucidation. Targeting BCAAs and other amino acids, such as methionine and lysine, holds promise in disrupting leukemia cell proliferation and promoting apoptosis. These findings underscore the potential of exploiting leucine metabolism and amino acid pathways for therapeutic benefits in AML.

Keywords: Acute Myeloid Leukemia (AML); Leucine deprivation; Branched-chain amino acids (BCAAs); Therapeutic benefits in AML

1. Introduction

Acute Myeloid Leukemia (AML) is a malignant hematological disorder characterized by the uncontrolled proliferation of myeloid progenitor cells in the bone marrow (1). Despite significant strides in leukemia classification and prognostication, overall survival rates for AML patients, especially in the elderly population, remain unsatisfactory (2). Conventional treatment modalities such as chemotherapy and hematopoietic stem cell transplantation often entail considerable limitations and adverse effects, necessitating the exploration of novel therapeutic strategies to improve patient outcomes and personalize treatment approaches in AML (3). In recent years, metabolic reprogramming has emerged as a promising avenue in cancer research, unveiling altered metabolic profiles in cancer cells to fuel their uncontrolled growth and proliferation (4). Notably, dysregulated amino acid metabolism, including perturbed leucine metabolism, has garnered substantial attention in the context of AML. Yet, the precise implications of leucine metabolism in AML and its potential impact on leukemic cells under leucine-deprived conditions remain incompletely understood (5).

Research evidence suggests that leucine metabolism is indeed dysregulated in AML. Enhanced expression of leucine transporters, particularly LAT1, has been observed in leukemic cells, signifying an augmented demand for leucine to support their heightened proliferation rates (6). Furthermore, alterations in the activity and expression of enzymes involved in leucine metabolism, such as BCAT1 and LHD, have been documented in AML, implicating leucine metabolism as a potential therapeutic target (7). The prospect of targeting leucine metabolism in AML holds promise. Strategies encompassing leucine deprivation, such as restricting dietary leucine intake or employing pharmacological inhibitors

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of leucine transporters or enzymes involved in leucine metabolism, have demonstrated encouraging outcomes in various cancer types (8). Notably, preclinical studies have indicated that leucine deprivation can effectively curb leukemic cell proliferation, induce cell cycle arrest, and promote the differentiation of leukemic cells (9). Additionally, when combined with conventional chemotherapy, leucine deprivation has shown synergistic effects, potentially enhancing therapeutic efficacy (10).

However, while the anti-leukemic effects of leucine deprivation appear promising, the intricate molecular mechanisms underlying this phenomenon necessitate further elucidation. It has been postulated that leucine deprivation may perturb the mTOR signaling pathway, a critical regulator of cell growth and proliferation, which is often hyperactivated in cancer cells, including leukemic cells. By attenuating mTOR signaling, leucine deprivation may induce cell cycle arrest and trigger cell death in leukemic cells, warranting comprehensive investigations to unravel these intricate molecular cascades. Beyond leucine metabolism, alterations in amino acid metabolism as a whole have been implicated in AML and other malignancies. Amino acids, the building blocks of proteins, participate in diverse cellular activities and metabolic pathways (11). Essential BCAAs, including valine, leucine, and isoleucine, are utilized in the tricarboxylic acid (TCA) cycle to compensate for glucose metabolism deficiencies. Targeting amino acid metabolism, as an essential sustenance for tumor cells, has demonstrated promise in inhibiting tumor growth (12). Metabolic starvation therapy, designed to limit the uptake of specific metabolites, such as certain amino acids, seeks to disrupt leukemia cell proliferation while exerting minimal toxicity on normal cells (13).

Among the BCAAs, leucine's co-amino acids, novel insights have emerged concerning their role in AML. Overexpression of BCAT1, responsible for BCAA metabolism, has been associated with poor prognosis in AML, further highlighting the significance of BCAAs in leukemogenesis. Additionally, targeting BCAT1 has demonstrated potential in promoting leukemia cell differentiation and restraining their growth, signifying a promising therapeutic avenue (14). Lysine, an essential amino acid, may regulate AML cell survival through redox metabolism reprogramming, with elevated lysine transporter expression associated with AML prognosis.

This study bears significant clinical implications for AML therapy by unraveling the role of leucine metabolism and other BCAAs in cancer cell viability. Understanding the molecular underpinnings of leucine deprivation's anti-leukemic effects can aid in the development of targeted therapies with improved efficacy and reduced side effects. Additionally, insights into the role of lysine and arginine in AML cell survival may lead to novel therapeutic strategies that target amino acid metabolism to combat leukemogenesis.

Overall, this research may pave the way for personalized treatment approaches and foster advancements in the field of metabolic reprogramming for cancer therapy. This study has several limitations. Firstly, cell-based assays may not fully represent the complexities of the in vivo tumor microenvironment, necessitating further validation using animal models. Secondly, the heterogeneity of AML patient samples and cell lines could affect the generalizability of the results. However, efforts will be made to reduce bias through rigorous experimental design and statistical analyses. Lastly, the precise mechanisms of amino acid metabolism in leukemogenesis may require additional investigations beyond the study's scope.

This study aims to investigate the implications of amino acid restriction, particularly leucine, on cancer cell viability, with a specific focus on Acute Myeloid Leukemia (AML). Through comprehensive examination of various cell lines, including MOLT4 and TK6 cells, we seek to discern the impact of leucine deprivation on cell survival using the MTS assay. Additionally, we aim to explore the dynamic response of TK6 frozen cells following thawing and prolonged culture to unravel the significance of leucine in supporting their viability. In our investigation of AML patient cells, we will consider diverse cell types and genotypes to elucidate potential variations in leucine dependency. Moreover, we aspire to delve into the molecular underpinnings of leucine deprivation's anti-leukemic effects, focusing on the mTOR signaling pathway and relevant cellular cascades involved in cell cycle regulation and survival.

2. Materials and Method

2.1. Tissue Culture

To initiate the cell splitting process, MOLT4/TK6 tumor cells were subjected to specific culture conditions involving RPMI 10% FBS supplemented with 2% L-Glutamine and 1% Penicillin-Streptomycin. Subsequently, the cells were incubated for a duration of 48 hours at a temperature of 37°C in a humidified atmosphere with 5% CO2. Following incubation, the cells were centrifuged at 900 rpm for 5 minutes, and trypan blue staining was employed for cell counting. During the 24-hour treatment phase of the experiment, the following procedures were executed. The cell count was determined using trypan blue staining. The treatment samples consisted of several control groups, including

a blank control, arginine-deprived group, lysine-deprived group, leucine-deprived group, as well as groups deprived of arginine, lysine, and leucine simultaneously. Each tube contained 2.6 million cells, which were subjected to microcentrifugation at 1.5 rpm for 5 minutes.

The cell lines were then seeded into 6-well white plates at a density of 2.6×10^5 cells per well for further treatment. After 24 hours, the tumor cells were once again subjected to microcentrifugation at 1.5 rpm for 5 minutes, followed by resuspension and transfer to their designated wells in a 96-well white plate. The plate was covered with foil paper and incubated for 2 hours at 37°C in a humidified atmosphere with 5% CO2. Finally, cell viability was assessed using an MTS assay.

2.2. Frozen Cells

To revive the frozen tumor cells, specifically TK6 cells, an aliquot of the frozen cells was re-suspended in 10% FBS serum. The suspension was then incubated at 37°C in a humidified atmosphere with 5% CO2 for a duration of 30 minutes. Following the incubation period, the cells underwent centrifugation at a speed of 500×g for 5 minutes. The cell pellet was carefully resuspended, and the cells were processed accordingly on Day 3, Day 6, Day 9, and Day 15, as required for TK6 cells. The resuspended cells were then incubated at 37°C in a humidified atmosphere with 5% CO2. During the treatment phase for the revived frozen cells, the cells were centrifuged once again at a speed of 500×g for 5 minutes. The cell pellet was resuspended in fresh media and used trypan blue, and the resulting suspension was transferred to 96-well white plates. Subsequently, the plates were incubated for 24 hours at 37°C in a humidified atmosphere with 5% CO2. After the 24-hour incubation, MTS assays were conducted, involving microcentrifugation at a speed of 500×g for 5 minutes.

2.3. Patient's Cells

Thawing and recovering cryopreserved cells is a critical step in cell-based research, particularly when working with tumor cells such as TK6. To initiate the thawing process, a mixture of 50% FBS/RPMI was utilized, providing essential growth factors for rapid energy uptake by the cells. Additionally, a higher concentration of 20% FBS/RPMI was prepared to ensure the cells' swift recovery. Both media solutions were prepared at a 1x concentration using ultrapure autoclaved sterile filtered water to maintain the integrity of cellular proteins. Careful attention was paid to the thawing procedure to prevent contamination and protect the delicate cells. The cryovial, retrieved from liquid nitrogen, was thawed in a 37°C water bath while continuously swirling to promote thorough mixing. The cell mixture was then transferred to a 15mL tube along with the remaining thawing medium, ensuring gentle mixing over a period of approximately 5 minutes. It was crucial to avoid rapid pipetting to prevent cell damage. The aim was to complete the thawing process within 10 minutes, allowing for the diffusion of dimethyl sulfoxide (DMSO) out of the cells and the uptake of nutrients.

After thawing, the cells underwent centrifugation at 200xg for 10 minutes. The supernatant was carefully removed, and the cells were resuspended in 1mL of 20% FBS/RPMI. Subsequently, 9mL of media was added and gently mixed. The cells were then subjected to another round of centrifugation at 300xg for 5 minutes, and the supernatant was discarded. To promote cell recovery and viability, the cells were resuspended in 10mL of 20% FBS/RPMI and incubated for 24 hours at 37°C with 5% CO2.

To assess cell viability, a Trypan blue assay was performed after the 24-hour incubation period. Additionally, 2×10^6 cells/mL were taken for further analysis. The cells were washed with PBS through centrifugation steps and subsequently resuspended in an appropriate buffer. Following the treatment phase, including control groups and specific conditions, the treated cells were transferred to 96-well white plates for subsequent cell counting and MTS assays. The MTS assay involved centrifugation at 500xg to facilitate accurate measurements.

3. Results and discussion

In this study, we investigated the impact of leucine, lysine, and arginine deprivation on the viability of MOLT4 and TK6 cells, two different cell lines. The MTS assay was employed to assess cell viability after subjecting the cells to various amino acid restrictions for 24 hours. Statistical analysis was conducted using ANOVA followed by the Tukey post hoc test to determine significant differences among the experimental conditions. First, we examined the effect of amino acid deprivation on MOLT4 cells. Leucine deprivation led to a significant decrease in cell viability to 58%, indicating the critical role of leucine in supporting the survival of these cells. Lysine deprivation also exerted a substantial influence, reducing viability to 55%. In contrast, arginine deprivation only caused a slight decrease in cell viability to 98%. When all three amino acids were deprived simultaneously, MOLT4 cell viability dropped to approximately 60%. These findings confirmed the impact of leucine, lysine, and arginine restriction on MOLT4 cell viability (Fig. 1).

Next, we investigated the effects of amino acid deprivation on TK6 cells. Similar to MOLT4 cells, leucine deprivation resulted in a significant decline in viability to 64%, highlighting the importance of leucine for TK6 cell survival. Lysine deprivation also had a notable impact, reducing viability to 64%. However, arginine deprivation only caused a modest decrease to 83% in TK6 cell viability. Simultaneous deprivation of all three amino acids resulted in a viability of approximately 80% compared to the control. Although the differences were not statistically significant, the trends indicated the potential role of these amino acids in supporting TK6 cell viability (Fig. 2). Furthermore, we examined the effects of leucine deprivation on TK6 frozen cells following thawing and 15-day culture. Leucine-deprived TK6 cells showed a significant decrease in viability, reaching approximately 65% (Fig 3). This finding suggested that leucine may play a role in supporting the viability of both TK6 frozen cells and normal TK6 cells (Fig. 3 and Fig. 4).

During the 15-day culture period, TK6 cells exhibited a gradual improvement in cell viability and metabolic activity. However, a temporary decline in viability was observed on day 9, potentially influenced by factors such as nutrient availability, waste accumulation, or cellular senescence. Nonetheless, by day 15, a significant recovery in cell viability and metabolic activity was observed, indicating the resilience of TK6 cells over time (Fig. 5). Moving on to AML patient cells, leucine deprivation had varying effects depending on the cell type and genotype. AML 112 and AML 116 patient cells showed a significant decrease in viability under leucine deprivation, emphasizing their dependence on leucine for growth and survival (Fig. 6A and Fig. 7A). In contrast, AML 139 patient cells displayed only a slight decrease in viability, suggesting a lesser impact of leucine deprivation on these cells (Fig. 7B). AML 152 and AML 154 patient cells exhibited notable reductions in viability, highlighting the sensitivity of these cells to leucine deprivation (Fig. 7C and 7D). These findings underscored the varying responses of AML patient cells to leucine availability.

We also investigated the impact of leucine deprivation on adherent and non-adherent AML patient cells, as well as cells with different genotypes. Adherent AML patient cells showed a decrease in viability to 85% under leucine deprivation, indicating the essential role of leucine as a critical nutrient for their maintenance. Non-adherent AML patient cells, on the other hand, displayed higher viability even in the absence of leucine, suggesting alternative metabolic adaptations (Fig. 6B). Genotype analysis of AML patient cells with the t(8;21) translocation and normal genotype cells showed contrasting responses to leucine restriction. AML cells with t(8;21) translocation displayed a significant decrease in viability, indicating their dependence on leucine for survival, while normal cells exhibited a modest increase in viability under leucine restriction (Fig. 8A and Fig. 8B). To assess the significance of these differences, an independent samples t-test was conducted, and the Levene's test confirmed no significant variance difference (p-value = 0.229), supporting the use of the unpaired t-test for viability comparison (Table 1).

3.1. The effect of leucine, lysine and arginine restriction on MOLT4 cells

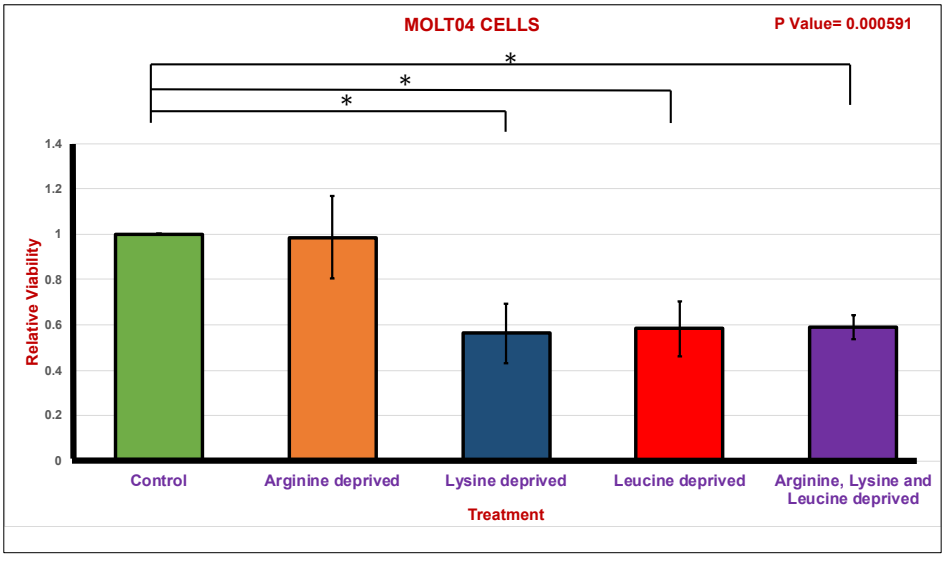

Figure 1 The effect of leucine, lysine and arginine restriction on MOLT4 cells(n=4).

Cells (2×10^6 ml) were treated for 24 hours with restriction of leucine, lysine and Arginine. Viability was assessed using an MTS assay in complete RPMI media. Error bars show standard deviation. P value is less than 0.05. ANOVA and Tukey post hoc justify, here * sample indicate that *<0.05.

3.2. The effect of leucine, lysine and arginine restriction on TK6 cells

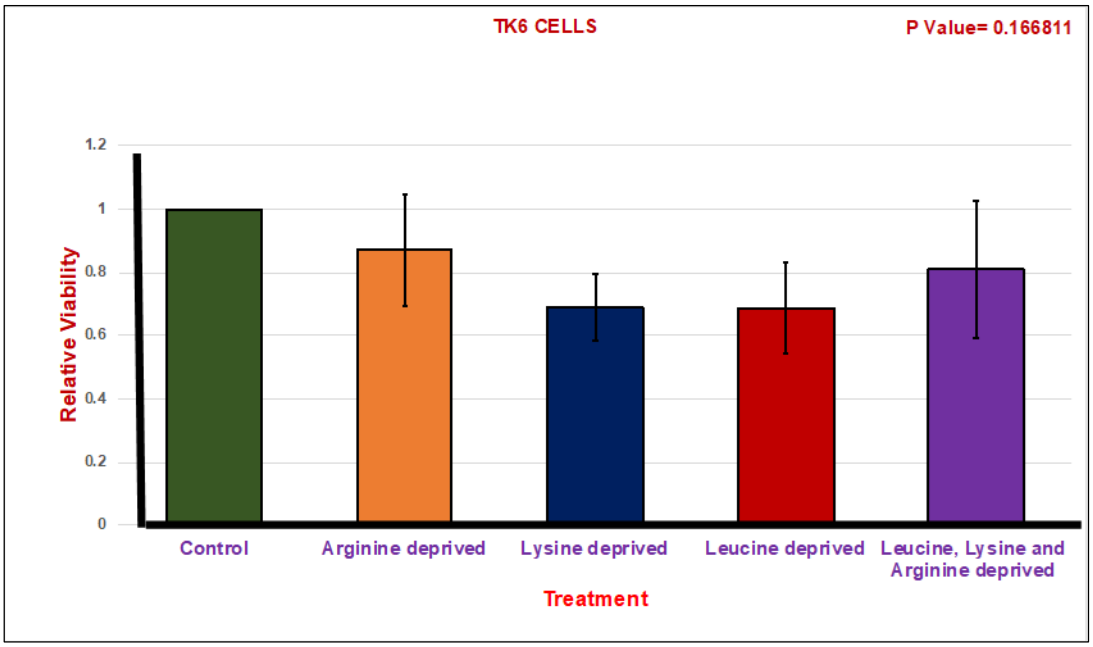

Figure 2 The effect of leucine, lysine and arginine restriction on TK6 cells(n=4).

TK6 cells (2×10^6 ml) were treated for 24 hours with deprivation of Leucine, Lysine and Arginine. Viability was assessed using an MTS assay in complete RPMI media. Error bars show standard deviation. TK6 cells p value is 0.166811, so it's not significant.

3.3. The effect of leucine deprivation on frozen TK6 cells

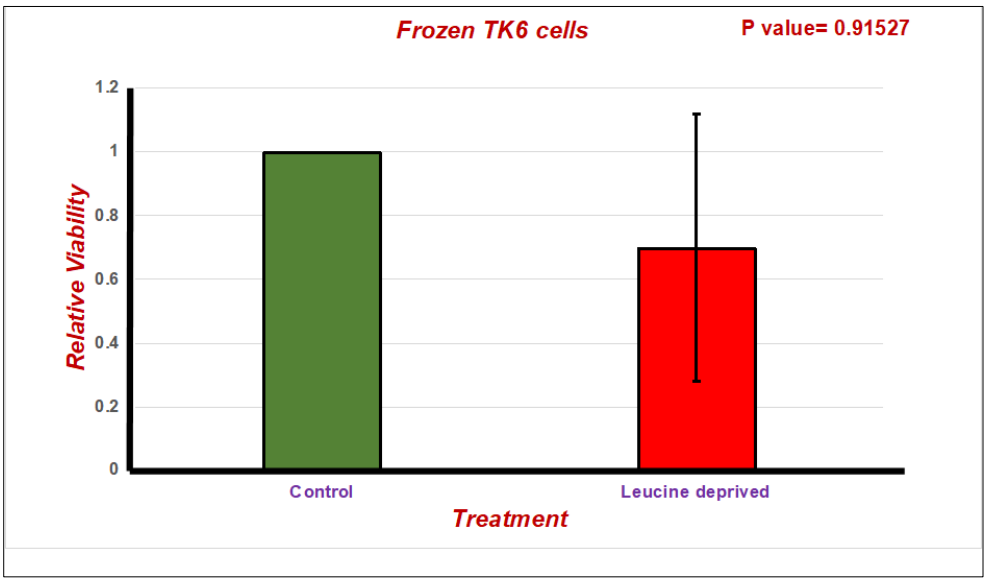

Figure 3 The effect of leucine on TK6 cells (Day 15 from frozen).

TK6 cells were treated for 24 hours with restriction of leucine. Viability was assessed using an MTS assay in complete RPMI media and error bars show standard deviation.

3.4. The effect of leucine deprivation on normal TK6 cells

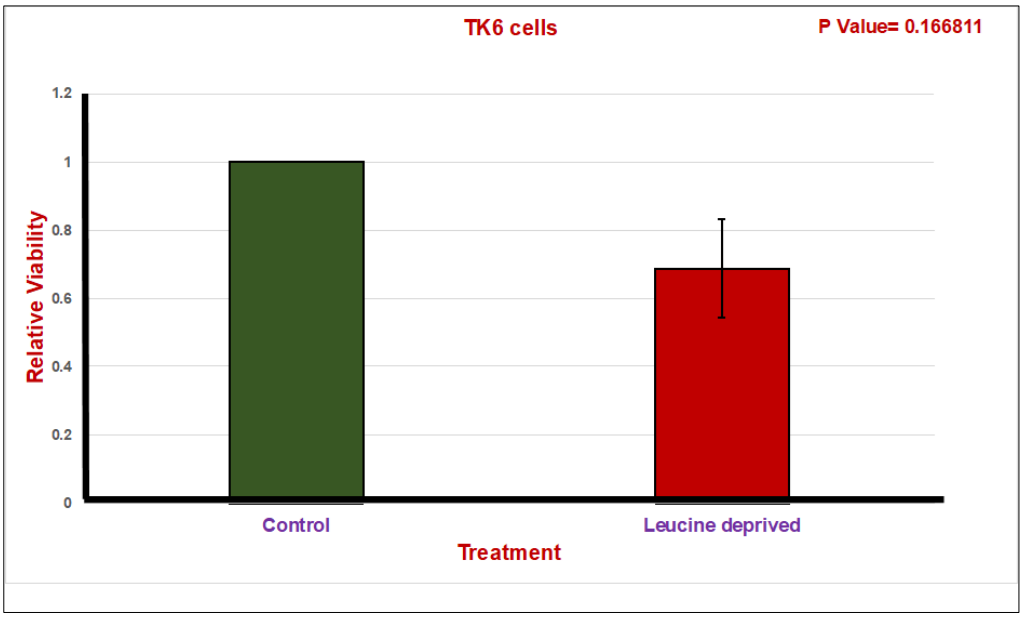

Figure 4 The effect of leucine on normal TK6 cells. TK6 cells were treated for 24 hours with restriction of leucine. Viability was assessed using an MTS assay in complete RPMI media and error bars show standard deviation.

3.5. The effect of All Amino Acid deprivation on frozen TK6 cells

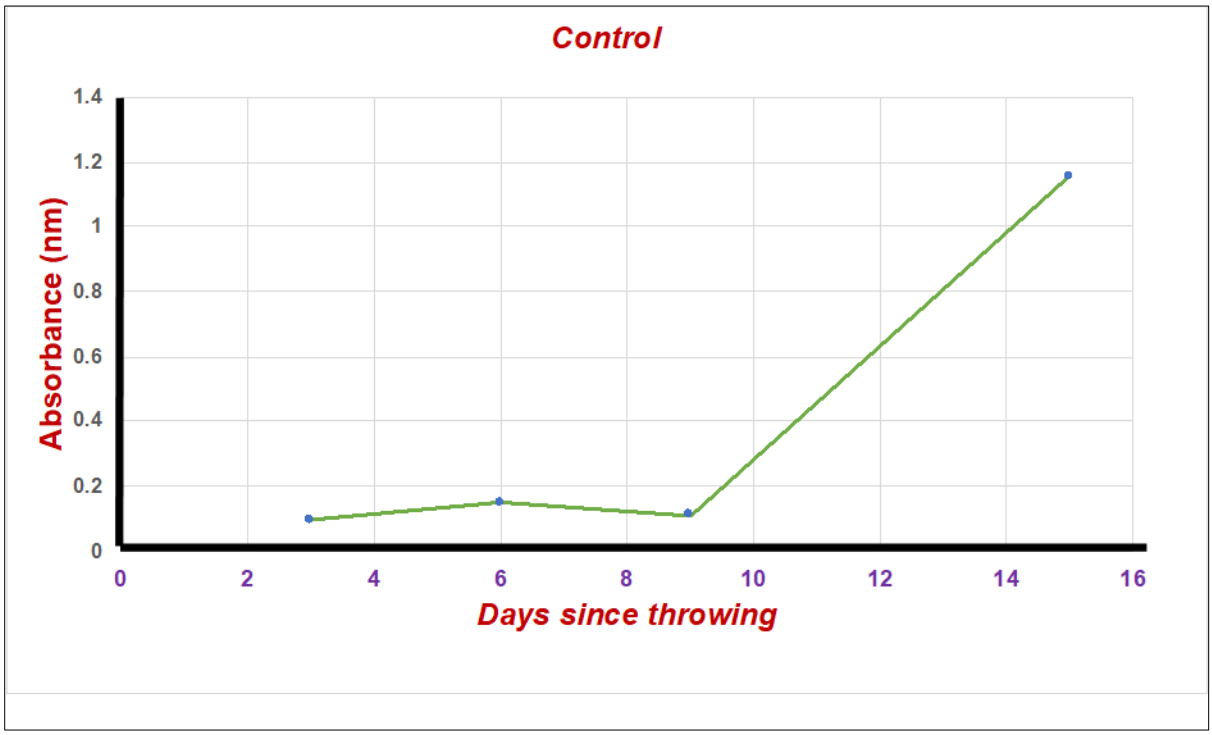

Figure 5 The effect of All Amino Acids on frozen TK6 cells (From Day3 to Day15).

TK6 cells were treated for 24 hours with restrictions of leucine. Viability was assessed using an MTS assay in complete RPMI media.

3.6. The effect of leucine deprivation on AML patient's cells (112)

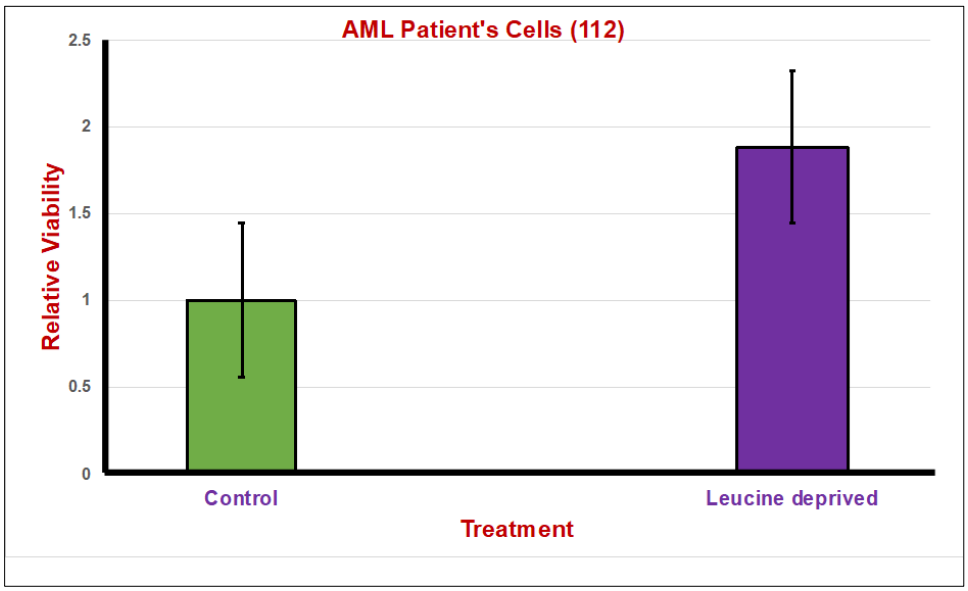

Figure 6A The effect of leucine on AML112 patient's cells. Cells were treated for 24 hours with restriction of leucine. Viability was assessed using an MTS assay in complete FBS/RPMI media and error bars show standard deviation.

3.7. The effect of leucine deprivation on AML patient's adherent cells (112)

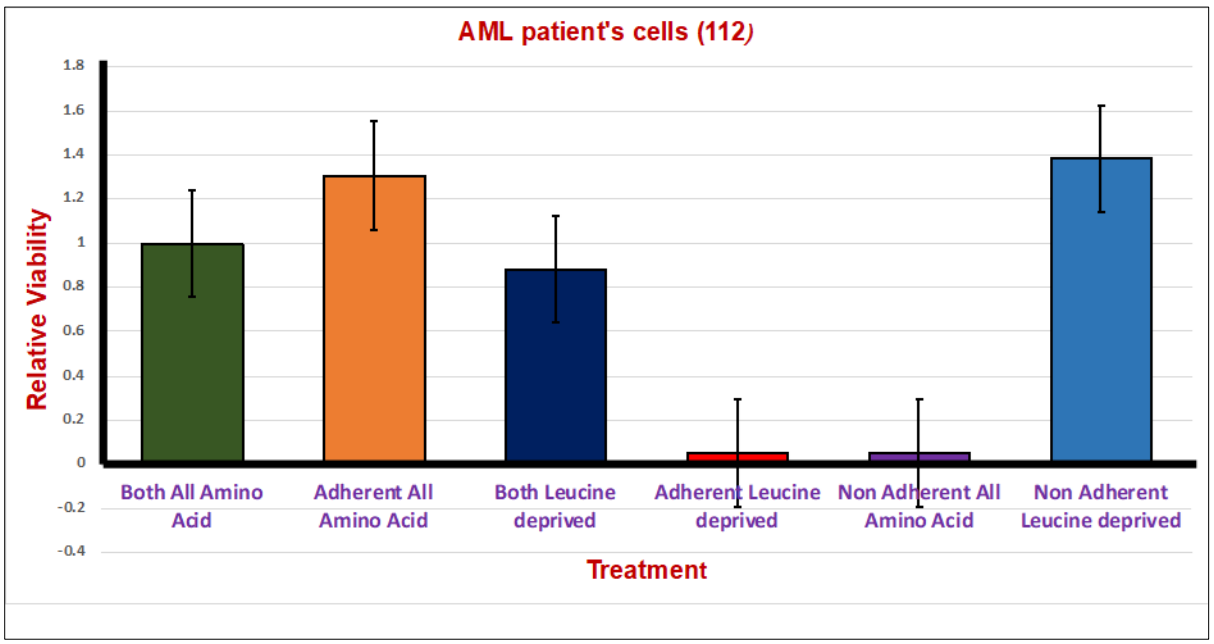

Figure 6B The effect of leucine on AML patient's cells with adherent cells.

Cells were treated for 24 hours with restriction of leucine. Viability was assessed using an MTS assay in complete FBS/RPMI media and error bars show standard deviation.

3.8. The effect of leucine deprivation on AML patient's cells (116)

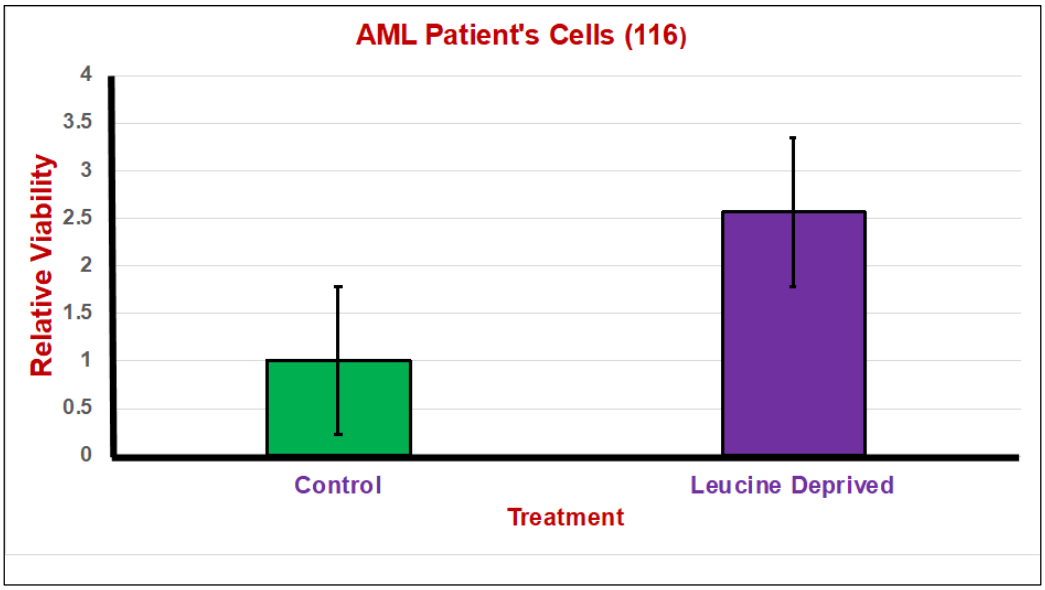

Figure 7A The effect of leucine on AML 116 patient's cells.

Cells were treated for 24 hours with restriction of leucine. Viability was assessed using an MTS assay in complete FBS/RPMI media and error bars show standard deviation.

3.9. The effect of leucine deprived on AML patient's cells (139)

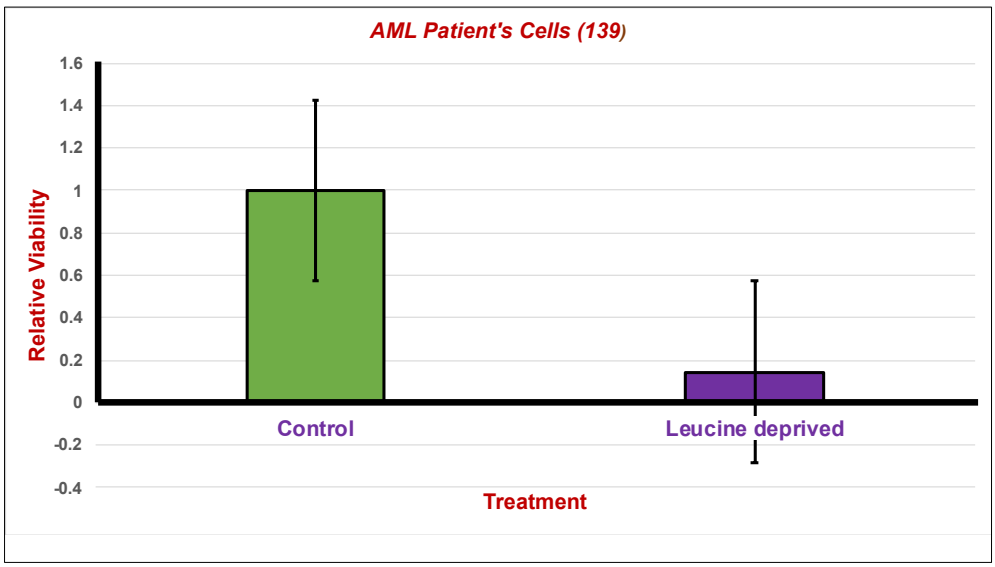

Figure 7B The effect of leucine on AML 139 patient's cells.

Cells were treated for 24 hours with restriction of leucine. Viability was assessed using an MTS assay in complete FBS/RPMI media and error bars show standard deviation.

3.10. The effect of leucine deprived on AML patient's cells (152)

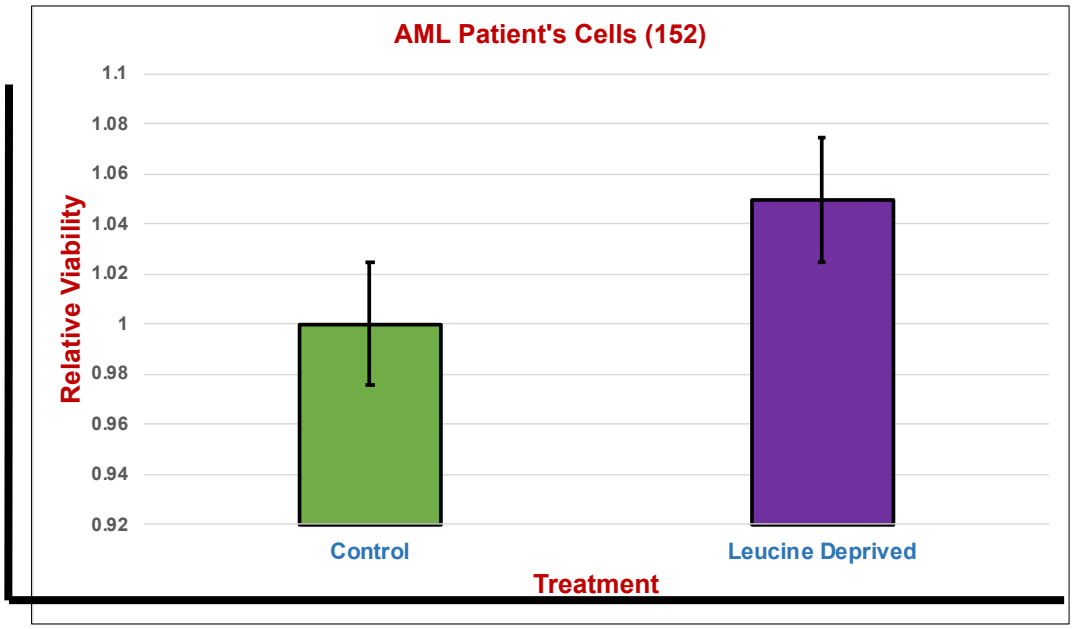

Figure 7C The effect of leucine on AML 152 patient's cells.

Cells were treated for 24 hours with restriction of leucine. Viability was assessed using an MTS assay in complete FBS/RPMI media and error bars show standard deviation.

3.11. The effect of leucine restriction on AML 154 patient's cells

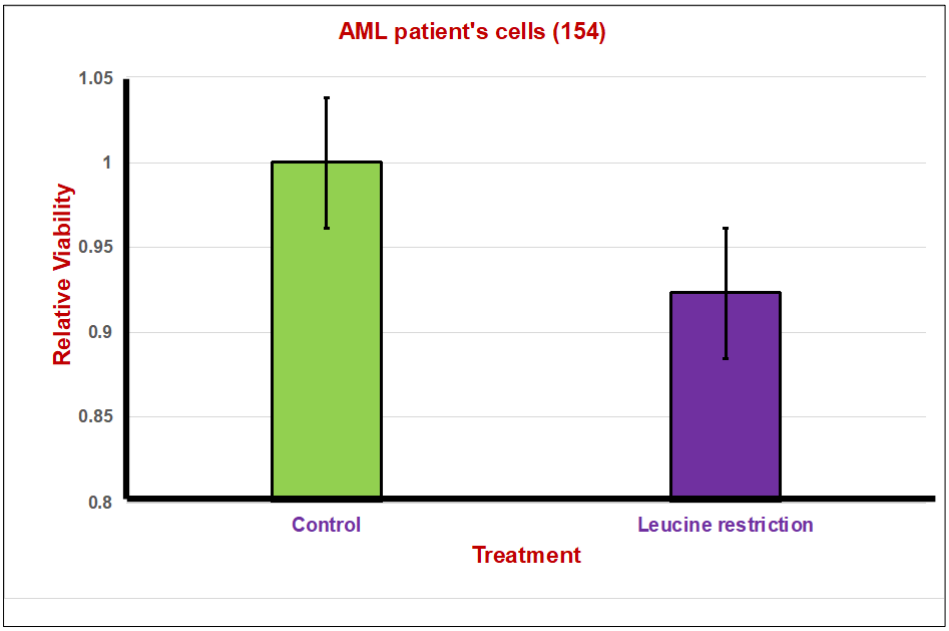

Figure 7D The effect of leucine on AML 154 patient's cells.

Cells were treated for 24 hours with restriction of leucine. Viability was assessed using an MTS assay in complete FBS/RPMI media and error bars show standard deviation.

3.12. The effect of leucine restriction on AML patient's genotype cells which translocation is t (8;21)

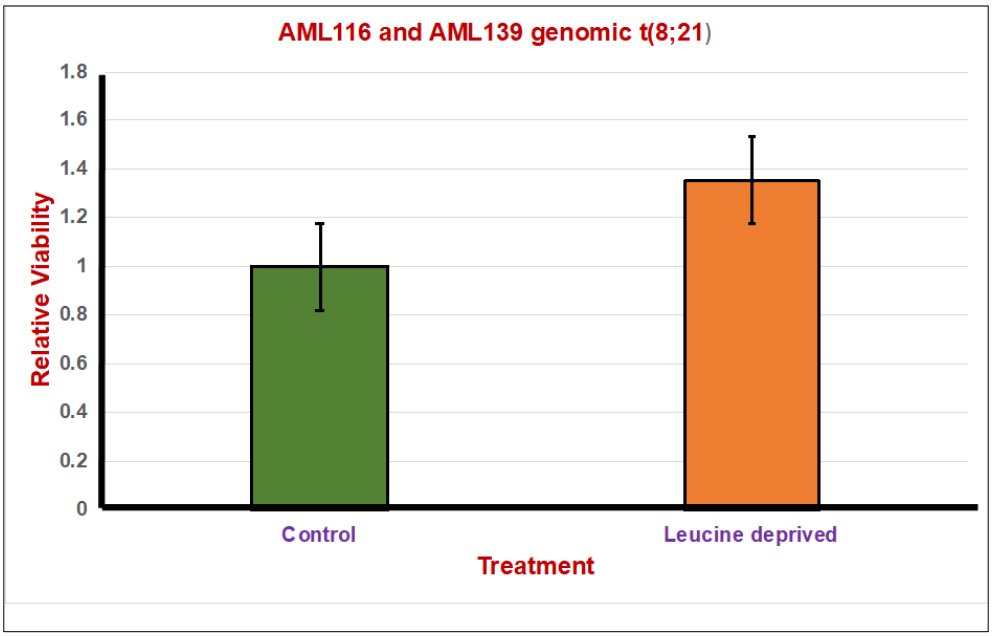

Figure 8A The effect of leucine on AML patient's cells.

Cells were treated for 24 hours with restriction of leucine. Viability was assessed using an MTS assay in complete FBS/RPMI media and error bars show standard deviation.

3.13. The effect of leucine restriction on AML patient's normal genotype cells

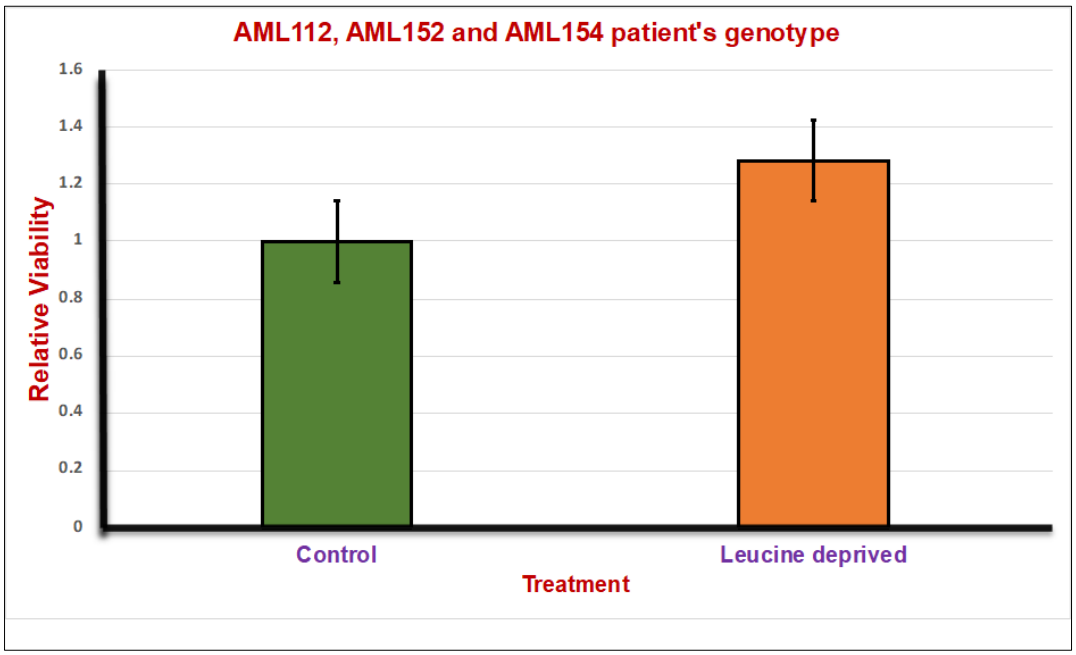

Figure 8B The effect of leucine on AML patient's cells.

Cells were treated for 24 hours with restriction of leucine. Viability was assessed using an MTS assay in complete FBS/RPMI media and error bars show standard deviation.

Table 1 Independent sample t Test (Unpaired t Test) for normal genotype (AML112, AML152, AML154) and genomic t(8;21)(AML116, AML139) AML patient's under leucine deprivation.

Independent Samples t Test (Unpaired t Test)

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance		Mean	Std. Error	95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Difference	Difference	Lower	Upper
Leucine deprivation	Equal variances assumed	2.270	.229	-1.132	3	.170	.340	-.673	.595	-2.566	1.219
	Equal variances not assumed			-.997	1.511	.226	.451	-.673	.675	-4.695	3.349

Leucine, an indispensable branched-chain amino acid, plays a critical role in cancer cell metabolism and various cellular processes, making it a potential therapeutic target in acute myeloid leukemia (AML) (15). Recent research has unveiled dysregulated leucine metabolism in AML, characterized by heightened expression of leucine transporters and altered enzyme activity.

3.14. Leucine deprivation

In a study assessing the impact of leucine deprivation on AML cell viability, both MOLT4 and TK6 cells exhibited a significant reduction in viability levels. Under leucine-restricted conditions, MOLT4 cell viability decreased to 58%, and TK6 cell viability reduced to 64% compared to control conditions. These results suggest that leucine metabolism is crucial for supporting the growth and survival of AML cells. Consequently, targeting leucine metabolism may hold promise as a personalized therapeutic approach to combat AML, potentially enhancing treatment efficacy and improving patient outcomes.

We investigated the dysregulated leucine metabolism in acute myeloid leukemia (AML) and its potential as a therapeutic target. Activation of the mechanistic target of rapamycin (mTOR) signaling pathway by leucine is crucial for tumor expansion and angiogenesis (16). Moreover, leucine's involvement in energy metabolism through the tricarboxylic acid (TCA) cycle and its regulatory role in autophagy further underscore its significance in leukemic cell biology. In leucine metabolism can lead to disruptions in critical cellular processes and contribute to uncontrolled leukemic cell growth (17). Notably, key enzymes involved in leucine metabolism, such as BCAT1 and LAP, have been associated with aggressive disease phenotypes and poor prognosis in AML (18). The solute carrier family 7-member 5 (SLC7A5) transporter, which plays a vital role in leucine uptake, exhibits high expression in AML and other cancers. Inhibition of SLC7A5 has been shown to impair leukemia cell proliferation and sensitize cells to targeted therapies, suggesting it as a potential therapeutic target (19). Targeting leucine metabolism and related pathways holds promise as a strategy to disrupt cancer cell metabolism and protein synthesis, offering potential avenues for therapeutic intervention.

In our investigation, we aimed to uncover the molecular mechanisms underlying dysregulated leucine metabolism in AML. By exploring the impact of leucine deprivation on cell viability in AML cell lines MOLT4 and TK6, we sought to gain insights into the significance of leucine in supporting leukemic cell survival. Additionally, we assessed the expression levels of key leucine-metabolizing proteins, including BCAT1, LAP, and SLC7A5, to understand their potential roles in leucine metabolism and leukemogenesis. Our findings shed light on the critical role of leucine metabolism in AML and its potential as a therapeutic target. Understanding the intricate molecular mechanisms of leucine metabolism dysregulation could pave the way for the development of targeted therapies that specifically disrupt the metabolic pathways supporting leukemic cell growth and proliferation. By elucidating the connections between leucine metabolism and AML pathogenesis, we aim to contribute to the advancement of personalized and effective treatment approaches for AML patients.

3.15. Arginine deprivation

The results of subjecting MOLT4 and TK6 cells to arginine deprivation indicate that arginine may play a critical role in supporting cell survival and maintaining optimal viability, particularly in TK6 cells. In the case of MOLT4 cells, a slight decrease in cell viability to 98% was observed, although this decrease was not statistically significant. This suggests that MOLT4 cells may have a partial dependency on arginine for their survival. On the other hand, TK6 cells exhibited a more

substantial decrease in cell viability to 83% upon arginine deprivation, indicating that TK6 cells are more reliant on arginine for their viability compared to MOLT4 cells. The contrasting responses of MOLT4 and TK6 cells to arginine deprivation highlight the potential heterogeneity in the metabolic dependencies of different cancer cell types, even within the same cancer type as AML. It is essential to further investigate the specific mechanisms through which arginine supports cell viability in each cell type. Understanding these mechanisms may unveil potential therapeutic implications for targeting arginine metabolism in AML and other cancers.

The findings may have clinical implications, as they suggest that some AML cells may be more sensitive to arginine deprivation than others. This could potentially be exploited in the development of personalized treatment approaches, tailoring therapies based on the metabolic characteristics of individual AML tumors. The study of arginine metabolism in cancer, particularly in acute myeloid leukemia (AML), has provided crucial insights into its role in disease pathogenesis and potential therapeutic strategies. A key observation in AML is the lack of argininosuccinate synthetase 1 (ASS1) expression, resulting in AML cells becoming dependent on external sources of arginine for survival (20). Exploiting this metabolic vulnerability, therapeutic approaches targeting arginine availability through enzymatic depletion or competitive inhibition have emerged as promising options. These strategies selectively induce cell death in AML cells while sparing normal cells, making them attractive for targeted therapies (21). The vulnerability of ASS1-deficient AML cells to the cytotoxic effects of arginine-degrading enzymes produced by immune cells opens new possibilities for immune-based strategies involving arginine depletion (22). However, the complex interplay between arginine metabolism and immune responses adds intricacies to potential therapeutic approaches. Ongoing clinical trials aim to investigate the clinical utility of manipulating arginine metabolism in leukemia patients, and understanding the mechanisms underlying arginine's effects on AML cells and its interaction with the immune system is crucial for optimizing personalized therapeutic interventions. The role of arginine metabolism extends beyond AML, encompassing various pathways that influence cellular processes crucial to cancer development and progression.

Targeting arginine metabolism has shown promise in cancer treatment, and understanding the intricate interplay between arginine metabolism, immune responses, and tumor biology holds the potential to further enhance therapeutic strategies (23). In AML, a network of proteins regulates arginine metabolism, including ASS1, arginase, and arginine transporters, providing insights into metabolic dependencies and vulnerabilities of AML cells and paving the way for the development of targeted therapies (24). The role of arginine in translation, post-translational modifications, nitric oxide synthesis, and polyamine synthesis further highlights its significance in cancer cell biology, emphasizing the need for continued research in this area.

3.16. Lysine deprivation

Lysine deprivation profoundly affects cell viability, leading to a significant reduction in MOLT4 cell viability to 55% (Fig. 1). This observation highlights the vital importance of lysine, an essential amino acid, in maintaining the survival and proliferation of MOLT4 cells. Similar results were observed in TK6 cells, with lysine deprivation resulting in a notable reduction in cell viability to 64% (Fig. 2). Lysine plays a critical role in diverse cellular functions, including protein synthesis and various metabolic pathways, emphasizing its indispensability for supporting the viability of both MOLT4 and TK6 cells. The results of subjecting MOLT4 and TK6 cells to lysine deprivation indicate that lysine is crucial for maintaining the survival and proliferation of these cells. Lysine is an essential amino acid, meaning that the cells cannot synthesize it on their own and rely on external sources for their lysine needs. When lysine is restricted from the cell culture medium, the cells are unable to access this critical amino acid, leading to a significant reduction in cell viability. The profound impact of lysine deprivation on cell viability in both MOLT4 and TK6 cells highlights the vital importance of lysine in supporting various cellular functions. Lysine is involved in protein synthesis, playing a fundamental role in building and maintaining cellular structures and functions.

In the context of acute myeloid leukemia (AML), lysine's multifaceted influence impacts critical aspects of leukemia and disease progression. Lysine's involvement in protein synthesis is particularly significant, as it facilitates cellular growth and proliferation, processes often dysregulated in leukemia (25). Furthermore, lysine participates in epigenetic modifications, where changes in lysine residues on histone proteins can influence gene expression and chromatin structure, contributing to leukemogenesis (26). Lysine metabolism is interconnected with various metabolic pathways, influencing energy production, redox regulation, and nucleotide synthesis in leukemic cells. Lysine availability and metabolism can also modulate immune responses in AML, potentially affecting disease progression and treatment outcomes (27).

Lysine's significance extends beyond AML, as it plays a complex and multifaceted role in cancer patients in general. Its pivotal role in protein synthesis enables the rapid cellular growth and proliferation observed in cancer cells, making it an attractive target for therapeutic interventions. Lysine also influences gene expression and chromatin structure

through post-translational modifications, contributing to the dysregulation of key cellular processes involved in cancer development and progression (28). Lysine's involvement in collagen synthesis and extracellular matrix remodeling influences tumor invasion, angiogenesis, and metastasis. The interplay between lysine metabolism and immune regulation adds another layer of complexity, as lysine availability can modulate immune cell function and anti-tumor immune responses. Additionally, lysine's role in metabolic pathways impacts energy production, redox regulation, and nucleotide synthesis, shaping cancer cell survival and proliferation (29). Understanding the intricate mechanisms by which lysine operates in cancer patients provides opportunities for targeted therapeutic interventions. Manipulating lysine availability, targeting lysine-related metabolic pathways, or considering lysine as an immunomodulatory factor may hold promise for optimizing treatment strategies and improving patient outcomes in cancer (30). The roles of lysine-metabolizing proteins in AML provide insights into disease biology and potential therapeutic targets. For instance, lysine-specific demethylase 1 (LSD1) and lysine dehydrogenase (LHD) impact lysine availability and metabolism within leukemic cells, making them potential targets for therapeutic interventions (31). The metabolism of lysine in cancer patients is pivotal for protein homeostasis and cellular processes. Lysine's involvement in protein synthesis, post-translational modifications, and interconnections with metabolic pathways underscores its significance in cancer cell biology. Dysregulation of lysine metabolism can impact cancer cell growth, proliferation, and survival. Targeting lysine metabolism and related pathways has emerged as a potential therapeutic strategy in cancer treatment (32).

3.17. Frozen cells

We investigated the impact of leucine deprivation on TK6 frozen cells that were thawed and cultured for 15 days, as well as on normal TK6 cells. Under normal growth conditions without leucine restriction, the control group of TK6 frozen cells displayed 100% relative viability, indicating their healthy state in complete RPMI media. However, when subjected to leucine deprivation, the viability of TK6 frozen cells decreased to approximately 65% (Fig. 3). Similarly, in normal TK6 cells, the control group maintained 100% viability, while cells subjected to leucine deprivation exhibited a reduction in viability to around 63% (Fig. 4). These findings highlight the critical role of leucine availability in sustaining TK6 cell viability and underscore the impact of leucine deprivation on both frozen and normal TK6 cells.

The viability of frozen TK6 cells was evaluated over a period of 15 days following thawing, focusing on the effect of all amino acids. At day 3 after thawing, the observed low absorbance value indicated a relatively low cell viability at the early stage of the culture. However, by day 6, there was a gradual improvement in cell viability, as evidenced by the increased absorbance value. This suggests an enhancement in the metabolic activity and proliferation of TK6 cells. Subsequently, on day 9, a decrease in the absorbance value was observed, indicating a decline in cell viability compared to day 6. Several factors, including nutrient availability, the accumulation of metabolic waste, or cellular senescence, might contribute to this decrease. Notably, by day 15, a significant increase in the absorbance value was noted, indicating a remarkable improvement in cell viability and metabolic activity compared to the earlier time points (Fig. 5). These findings highlight the dynamic nature of cell viability in frozen TK6 cells and underscore the complex interplay between amino acid availability and cellular responses during the culture period. Further investigations are needed to elucidate the underlying mechanisms governing these observations and their implications for optimizing cell viability in frozen TK6 cells.

The study aimed to explore the behavior and potential applications of frozen TK6 cells, particularly in relation to AML. The findings revealed that frozen TK6 cells, upon thawing and culture, exhibited dynamic characteristics over time. They demonstrated an initial period of low cell viability on day 3 after thawing, which gradually improved by day 6 as indicated by increased metabolic activity and proliferation. However, a subsequent decline in cell viability was observed on day 9, potentially influenced by various factors such as nutrient availability, accumulation of metabolic waste, or cellular senescence. Notably, by day 15, there was a significant improvement in cell viability and metabolic activity.

3.18. AML Patient’s cells

The impact of leucine deprivation on the viability of AML patient's cells was investigated in this study. Different restrictions of leucine were applied to AML patient's cells for a 24-hour duration, and their viability was assessed. The control group, representing cells cultured under normal conditions without leucine deprivation, displayed 100% relative viability. However, upon leucine deprivation, the viability of AML patient's cells decreased significantly, surpassing 180%. It is noteworthy that the term "more than 180%" signifies a substantial reduction in viability beyond that of the control group (Fig. 6B).

In the case of adherent AML patient's cells, the control group, cultured with all amino acids, exhibited 100% relative viability. However, when subjected to leucine deprivation, the viability of adherent AML patient's cells decreased to 85%. This decline in viability upon leucine deprivation indicates a dependence on leucine availability for optimal growth

and survival. Conversely, non-adherent AML patient's cells cultured with all amino acids displayed viability exceeding 120%. Interestingly, when subjected to leucine deprivation, non-adherent AML patient's cells exhibited an increase in viability to nearly 140% (Fig. 6B). These observed differences between adherent and non-adherent AML patient's cells in response to leucine deprivation highlight the potential influence of cellular context on the cellular response to leucine availability. In AML patient's cells from different individuals (e.g., AML 116, AML 139, AML 152, and AML 154), leucine deprivation resulted in varying effects on cell viability. While the control groups of these cells displayed 100% viability under normal conditions, leucine deprivation led to substantial decreases in viability, ranging from more than 250% in AML 116 cells to less than 20% in AML 139 cells. Notably, AML 152 cells exhibited a significant drop in viability to 150%, while AML 154 cells experienced a notable decrease in viability to approximately 92% upon leucine deprivation.

3.19. Genotype

The effect of leucine restriction was examined on AML patient's genotype cells carrying a specific translocation, t(8;21), with a focus on AML116 and AML139 cells. These cells were exposed to different levels of leucine restriction for 24 hours. The control group, representing AML patient's genotype cells cultured without leucine restriction, demonstrated 100% relative viability, indicating their ability to maintain optimal viability under normal growth conditions. However, when the AML patient's genotype cells with the t(8;21) translocation were subjected to leucine restriction, a decrease in viability was observed, with viability reaching approximately 130% compared to the control. This reduction in viability suggests that leucine availability may play a role in supporting the survival and growth of AML patient's genotype cells carrying the t(8;21) translocation. The specific genetic alteration conferred by the translocation may influence the cellular response to leucine restriction, highlighting the need for further investigation into the underlying mechanisms involved (Fig. 8A).

Similarly, the effect of leucine restriction was assessed in AML patient's normal genotype cells, specifically AML112, AML152, and AML154 cells. These cells were exposed to different levels of leucine restriction for 24 hours, and their viability was determined using an MTS assay in complete FBS/RPMI media. The control group, consisting of AML patient's normal genotype cells cultured without leucine restriction, demonstrated 100% relative viability, indicating their ability to maintain optimal viability under normal growth conditions. Interestingly, when subjected to leucine restriction, a slight increase in viability was observed, with viability reaching approximately 130% compared to the control (Fig. 8B). These findings suggest that leucine restriction may have a different impact on AML patient's normal genotype cells compared to those with the t(8;21) translocation.

4. Conclusion

The significance of leucine, arginine, and lysine metabolism in acute myeloid leukemia (AML) was investigated. Leucine deprivation led to a substantial reduction in AML cell viability, potentially through perturbations in mTOR signaling and energy metabolism. Arginine and lysine deprivation also impacted cell viability, highlighting their importance in AML cell survival and proliferation. The findings suggest that targeting these metabolic pathways could offer promising therapeutic strategies for AML treatment. Additionally, frozen TK6 cells demonstrated dynamic characteristics, presenting a potential model for investigating AML cell viability and metabolic dependencies.

Compliance with ethical standards

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Disclosure of conflict of interest

There are no conflicts of interest.

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