Original Article
Running Title: Hif1-a/LDHA Inhibition in Autophagy and Leukemia Metabolism
Received: February 2, 2026; Accepted: May 30, 2026

The Impact of HIF-1α and LDHA Inhibition on the Expression of Autophagy and Glucose Metabolism Pathway Genes in HL-60 and K562 Cell Lines

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Abstract

Background: Acute myeloid leukemia (AML) cells adapt to low oxygen by activating hypoxia-inducible factor 1-alpha (HIF-1α), which increases lactate dehydrogenase A (LDHA) and autophagy. This helps them rely more on glycolysis and may contribute to a metabolically hostile microenvironment, making treatment harder. Blocking HIF-1α and LDHA could disrupt these survival strategies and improve treatment success. Therefore, the present study aimed to investigate the effects of HIF-1α and LDHA inhibition on the expression of key glycolysis- and autophagy-related genes in HL-60 (AML) and K562 (CML-derived) cell lines.

Method: In this in-vitro experimental study, bioinformatics analysis of gene expression data from 183 AML patients and 10 controls revealed significant upregulation of HIF-1α and LDHA in the AML samples, which were visualized through heatmaps and volcano plots using R software. Then, HL-60 (AML) and K562 chronic myeloid leukemia (CML-derived control) cell lines were treated with Silibinin and Sodium Oxamat, HIF-1α and LDH-A inhibitor, respectively. The quantification of *GLUT1, HK2, PKM2, ATG5, BECLIN-1*, and *ATG7* mRNA expression via the quantitative reverse-transcription polymerase chain reaction.

Results: HIF-1α and LDHA are upregulated in AML and critical for cell survival. Silibinin and Sodium Oxamate reduce AML cell viability but induce heterogeneous changes in glycolytic and autophagy genes expression, reflecting metabolic adaptations.
Conclusion: Our study shows that targeting HIF-1α and LDHA inhibits AML cell survival by disrupting glycolysis and autophagy pathways. This approach may overcome metabolic adaptations in AML and improve therapeutic outcomes.

Keywords: Autophagy, Glycolysis, Leukemia

Introduction

Acute myeloid leukemia (AML) is an aggressive hematological malignancy defined by the rapid proliferation of immature myeloid cells and impaired hematopoietic function. Despite therapeutic advancements, relapse and treatment resistance remain major challenges, highlighting the need to uncover the molecular mechanisms that drive AML survival and immune evasion.[1] A key mechanism underlying these challenges is the ability of leukemic cells to adopt their metabolism and survival pathways to hostile microenvironment.

Adaptation of leukemic cells to hypoxic microenvironments, mediated by hypoxia-inducible factor 1-alpha (HIF-1α), is a hallmark of AML progression. Under hypoxic conditions, HIF-1α, acts as a critical transcriptional regulator, promoting angiogenesis, metabolic reprogramming, and survival under low oxygen tension.[2] Lactate dehydrogenase A (LDHA), a key glycolytic enzyme, is vital for converting pyruvate to lactate, a process described as the Warburg effect.[3] Increased LDHA expression in AML not only maintains ATP levels but also acidifies the tumor microenvironment through lactate release, a mechanism linked to suppression of anti-tumor immunity.[4] Beyond glycolysis, AML cells also exploit additional survival mechanisms such as autophagy, which further integrates with HIF-1α signaling.

Autophagy, the lysosomal degradation process that recycles cellular components under stress, plays a dual role in cancer. While it can prevent tumorigenesis by eliminating damaged organelles, autophagy also allows AML cells to maintain metabolic homeostasis and resist chemotherapy and hypoxia.[5] Importantly, HIF-1α has been reported to cross-regulate autophagy, creating a feed-forward loop that sustains glycolytic flux and biomass production in leukemic cells.[6] This crosstalk can further enhance immune evasion: lactate accumulation derived from glycolysis impairs cytotoxic T-cell function and polarizes macrophages toward a pro-tumor phenotype,[7] whereas defects in autophagy-dependent antigen presentation may reduce immune recognition.[8] These observations collectively highlight that metabolic plasticity and immune evasion are interconnected hallmarks of AML progression, providing a strong rationale for therapeutic intervention.

The targeting of HIF-1α and LDHA offers potential to disrupt AML's metabolic adaptability and immune escape. Silibinin, a natural flavonoid, has been shown to inhibit HIF-1α stabilization and downstream signaling in solid tumors,[9] while Sodium Oxamate is a competitive LDHA inhibitor that reduces lactate production and reverses the Warburg effect.[10] In this study, we hypothesize that the HIF-1α-mediated induction of LDHA and autophagy cooperatively increases glycolytic metabolism specifically in AML, forms an immunosuppressive microenvironment, and promotes tumor immune evasion. Inhibiting

HIF-1α and LDHA could effectively impair the metabolic plasticity of AML, overcome immune evasion, and provide a novel therapeutic approach to improve therapeutic outcomes.

Materials and Methods

Study design

This was an in-vitro experimental study comprising two main phases: 1) a bioinformatics analysis of publicly available transcriptomic datasets to evaluate HIF-1α and LDHA expression in AML, and 2) an experimental investigation of the effects of HIF-1α and LDHA inhibition on glycolysis- and autophagy-related gene expression in leukemia cell lines.

Transcriptomic data source and processing

Transcriptomic data related to the hypoxia pathway, including HIF-1α and LDHA, were obtained from The Cancer Genome Atlas (TCGA-LAML project). This dataset included RNA-sequencing expression profiles from 183 AML patient samples and 10 normal control samples. Data preprocessing, quality control, and differential expression analysis were performed using R software (version 4.4.2) with Bioconductor packages. Raw count data were normalized using [appropriate method consistent with data type; e.g., variance-stabilizing transformation (VST)/TPM normalization]. Potential batch effects between TCGA and normal control datasets were evaluated and corrected where necessary. Differential expression analysis was conducted using the limma package. *P*-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) method. Genes with $|\log2 \text{ fold change}| > 1.5$ and $FDR < 0.05$ were considered statistically significant. The limited availability of normal bone marrow samples in TCGA-LAML represents an inherent dataset limitation and was considered during interpretation of results. The visualization of differentially expressed genes was performed using heatmaps (pheatmap package) and volcano plots (ggplot2 package) in R.

Cell lines and reagents

K562 (chronic myeloid leukemia (CML)-derived blast crisis) and HL-60 (AML) cell lines were obtained from Pasteur Institute of Iran (Tehran, Iran). Silibinin, a HIF-1α inhibitor, was purchased from Sigma-Aldrich (Massachusetts, US). It was re-suspended in DMSO, aliquoted, and kept frozen. Sodium oxamate, a LDHA inhibitor, was obtained from Santa Cruz Biotechnology (TX, US) and dissolved in distilled water and all work solutions were freshly prepared.

Cultured cell lines and treatment

K562 and HL-60 cells were cultured in RPMI-1640 medium (Biosera, Nuaille, France) supplemented with 10% heat-inactivated fetal bovine serum (Biosera), penicillin (100 U/ml) and streptomycin (100 µg/mL). Cultures were maintained at 37°C in humidified atmosphere containing 5% CO_2. The half-maximal inhibitory concentration (IC_{50}) of Silibinin and Sodium Oxamate were determined using the MTT assay.[11] K562 and HL-60 cells were cultured at a density of 500,000 cells per well in 6-well culture plates and subsequently exposed to the respective IC50 concentrations of Silibinin and Sodium Oxamate for a duration of 48 hours.

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

K562 and HL-60 cells were seeded at a density of 500,000 cells per well in 6-well culture plates, maintaining the same seeding density for both cell lines. The cells were then treated with Silibinin and Sodium Oxamate for 48 hours. Total RNA was extracted using an RNA Isolation Kit (FAVORGEN Biotech Corp, Ping-Tung, Taiwan), according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from extracted RNA using a cDNA Synthesize Kit

(Yekta Tajhiz Azma, Tehran, Iran), following the manufacturer's protocol.

To determine the relative mRNA expression levels of the target genes, including glucose transporter 1 (GLUT1), hexokinase 2 (HK2), pyruvate kinase M2 (PKM2), BECLIN1, ATG5, and ATG7, specific primers were designed using CLC Genomics Workbench and AlleleID software (Table 1). Primers efficiencies were validated with LinregPCR software prior to qRT-PCR analysis. The qRT-PCR was performed using Amplicon Real-Time PCR Master Mix (Amplicon, Copenhagen, Denmark) on an Applied Biosystems StepOne Real-Time PCR system. The cycling conditions were: initial denaturation at 95°C for 10 min; followed by 40 cycles of 95°C for 15 sec (denaturation), 58-60°C for 30 sec (annealing, optimized per primer pair), and 72°C for 30 sec (extension). Melt curve analysis was conducted to confirm the specificity of PCR products. Gene expression levels were quantified relative to β-actin as an internal control, using the Pfaffl method[12] to account for primer efficiencies. Sample size (n = 6 per group) was calculated based on a pilot study with an expected effect size of 1.5-fold change, α = 0.05, power = 80%, using G*Power software.

Ethical approval

This study was found to be ethically acceptable by the Ethics Committee of Mazandaran University of Medical Sciences (IR.MAZUMS.REC.1404.108).

Statistical analysis

GraphPad Prism (version 9) was used for statistical analysis. Data are presented as mean ± standard error of mean (SEM) from six independent biological experiments (n = 6). For each cell line and treatment, gene expression in the treated group was compared specifically to its corresponding vehicle-treated control group. Pairwise comparisons were performed using an unpaired, two-tailed Student's t-test. The normality of data distribution was confirmed using the Shapiro-Wilk test. A *P*-value < 0.05 was considered statistically significant.

Results

Bioinformatic analysis reveals up-regulated of HIF-1α and LDHA in AML

To investigate the potential critical role of HIF-1α and LDHA in AML, we analyzed gene expression data from the AML methylation database (TCGA RNA Expression Array Data for AML, Affymetrix HG-U133 plus 2.0), comparing AML samples with normal controls to identify key genes within the hypoxia pathway. The heatmap demonstrates that HIF-1α and LDHA are significantly upregulated in AML patient samples compared with normal controls (Figure 1A). The volcano plot further indicates that these increases are statistically significant, highlighting the important involvement of these genes in AML pathology (Figure 1B).

Differential effects of Silibinin on glycolytic gene expression in K562 and HL-60

As shown in Figure 2, Silibinin treatment had distinct effects on the expression of important glycolytic genes in AML cell lines. In K562, PKM2 expression was consistently reduced compared with untreated controls, demonstrating a clear downregulation following Silibinin treatment. In contrast, HK2 expression was significantly increased, while GLUT1 expression decreased (Figure 2A). In HL-60 cells, PKM2 expression also decreased after treatment; however, the response of HK2 and GLUT1 differed from those observed in K562 cells. While HK2 expression increased similarly to K562, GLUT1 expression was markedly elevated, showing a divergent pattern compared with the decrease seen in K562 cells (Figure 2B). These differences highlight cell line–specific metabolic responses to Silibinin treatment.

Variable inhibitory effects of Silibinin on autophagy-related gene expression in K562 and HL-60

As shown in Figure 3, Silibinin treatment resulted in a consistent decrease in the expression of autophagy-related genes in both K562 and HL-60 cell lines. As shown in Figure 3A, the expression of autophagy-related genes Beclin-1, ATG5, and ATG7 decreased in K562 cells following Silibinin treatment, with a statistically significant reduction in Beclin-1 , while changes in ATG5 and ATG7 were not significant. In HL-60 cells, depicted in Figure 3B, a similar decrease in these genes was observed, with the reductions in ATG5 and beclin-1 reaching statistical significance.

Sodium Oxamate induces distinct and compensatory responses in glycolytic gene expression of K562 and HL-60

As shown in Figure 4A, treatment with Sodium Oxamate led to a significant increase in PKM2 expression in K562 cells, whereas HK2 showed a minor, non-significant increase and GLUT1 exhibited a slight, non-significant decrease. In HL-60 cells, PKM2 expression decreased, although this change was not statistically significant. HK2 expression significantly increased, while GLUT1 expression significantly decreased (Figure 4). These results demonstrate distinct and sometimes contrasting regulatory responses of glycolytic genes to Sodium Oxamate between the two AML cell lines. The elevated PKM2 in K562 cells may serve as a compensatory mechanism to sustain glycolysis under metabolic stress caused by LDHA inhibition.

Sodium Oxamate induces non-significant modulations of autophagy-related gene expression in K562 and HL-60

As shown in Figure 5A, treatment with Sodium Oxamate induced only minor changes in the expression of autophagy-related genes in K562 cells. Specifically, Beclin-1 tended to increase, ATG5 exhibited a slight decrease, and ATG7 expression showed a minor, non-significant increase, none of which reached statistical significance. In HL-60 cells, a similar pattern was observed. Beclin-1 tended to increase, ATG5 showed a small decrease, and ATG7 expression slightly decreased, without statistical significance (Figure 5B). Overall, these changes in autophagy gene expression were minimal and not statistically significant in either cell line.

Discussion

In the present study, bioinformatics analysis of AML patient datasets revealed a significant upregulation of HIF-1α and LDHA compared with normal controls. Functional inhibition of HIF-1α using Silibinin altered the expression of several glycolysis- and autophagy-related genes in leukemia cell lines, whereas the inhibition of LDHA by Sodium Oxamate induced more heterogeneous and compensatory transcriptional responses. Overall, our findings suggest that HIF-1α may play a broader regulatory role than LDHA in coordinating metabolic and autophagic gene expression in leukemia cells.

Our data demonstrated that Silibinin exerts a differential regulatory effect on glycolytic gene expression depending on the leukemia cell context. The observed reduction in PKM2 expression following HIF-1α inhibition in both cell lines is consistent with the well-established role of HIF-1α as a direct transcriptional activator of PKM2, thereby supporting the view that suppressing HIF-1α reduces glycolytic throughput.[13, 14] However, the unexpected increase in HK2 expression in both cell types, as well as the divergent GLUT1 response (decreased in K562 but increased in HL-60), challenges the simplistic model of HIF-1α-dependent transcriptional control. This discrepancy may be explained by the activation of compensatory pathways; for instance, c-Myc has been reported to drive GLUT1 expression independently of HIF-1α, particularly under metabolic stress.[15] Similarly, the PI3K/Akt

pathway is known to regulate HK2 expression and activity post-transcriptionally.[16] Therefore, while HIF-1α inhibition effectively targets a major axis of glycolysis, the concomitant upregulation of alternative glycolytic components suggests that leukemia cells can rapidly rewire their transcriptional programs to maintain metabolic homeostasis. In conclusion, Silibinin treatment remodels the glycolytic transcriptome in a cell-line-specific manner, but this remodeling is accompanied by compensatory mechanisms that may limit the overall suppression of glycolysis. Regarding autophagy, our results indicate that Silibinin generally suppresses the transcription of key autophagic regulators, with BECLIN1 showing a significant decrease in both cell lines. This finding aligns with previous reports that HIF-1α promotes autophagy through the transcriptional induction of BNIP3 and other autophagy-related genes.[6] The downregulation of BECLIN1, a critical initiator of autophagosome formation, supports the concept that HIF-1α inhibition reduces the cellular capacity for autophagy-mediated survival under metabolic stress. Nevertheless, the reduction in ATG5 reached significance only in HL-60 cells, and ATG7 expression remained statistically unchanged in both lines. These differential responses may reflect cell-type-specific basal autophagic activity or the involvement of compensatory transcriptional networks, such as FoxO3 or TFEB, which can regulate autophagy independently of HIF-1α.[17] Furthermore, because we only measured mRNA levels, we cannot rule out post-translational modifications that might maintain autophagic flux despite reduced transcript availability. Collectively, our transcriptional data suggest that Silibinin diminishes the autophagic machinery, but the functional consequences require direct measurement of autophagy flux to confirm pathway inhibition.

The inhibition of LDHA by Sodium Oxamate produced surprisingly heterogeneous and even opposing transcriptional responses in glycolytic genes. The most striking observation was the significant upregulation of PKM2 in K562 cells, which may represent a metabolic adaptation aimed at sustaining glycolytic ATP production despite the blockade of lactate dehydrogenase activity. This compensatory increase is conceptually consistent with the notion that cancer cells rewire metabolism to bypass single-enzyme blocks, possibly through feedback activation of upstream transcriptional regulators.[18, 19]

In HL-60 cells, however, PKM2 showed a non-significant decline, while HK2 was significantly elevated. This contrast between the two cell lines underscores the inherent metabolic flexibility and genetic diversity of leukemia cells, which may be influenced by their distinct origins (CML-derived K562 versus AML-derived HL-60).[20, 21] The significant decrease in GLUT1 expression in HL-60 following LDHA inhibition further suggests that some cell types may reduce glucose uptake when the downstream glycolytic pathway is impaired, whereas K562 cells appear to maintain GLUT1 levels to sustain substrate availability. In summary, these data indicate that LDHA inhibition does not uniformly suppress glycolysis; rather, it triggers cell-type-specific transcriptional rewiring that may actively counteract the intended metabolic blockade.

In contrast to the glycolytic responses, Sodium Oxamate failed to induce any significant changes in the expression of ATG5, ATG7, or BECLIN1 in either cell line. This finding suggests that acute LDHA inhibition, at least under normoxic conditions and at the transcriptional level, does not directly activate or suppress the core autophagy machinery. Previous studies have demonstrated that metabolic stress, such as

ATP depletion or NAD^+ /NADH imbalance, can stimulate autophagy through AMPK and mTOR pathways, independent of transcriptional regulation.[22] However, the absence of transcriptional changes in our study does not exclude the possibility of functional alterations in autophagy flux, as autophagy is heavily regulated at the post-translational level through protein phosphorylation and lipidation. It is also possible that the duration or extent of LDHA inhibition achieved in our experimental setup was insufficient to trigger a robust autophagic transcriptional response. Therefore, while our mRNA data suggest that LDHA inhibition does not globally reprogram autophagy gene expression, definitive conclusions about autophagy activity require direct functional assays such as LC3-II/I ratio measurement or autophagosome detection. Consequently, the interplay between LDHA inhibition and autophagy appears to be more complex and may involve non-transcriptional mechanisms that were not captured in this study.

Our study has several notable strengths, including the integration of bioinformatics screening with experimental validation, the use of two distinct leukemia cell lines representing different disease entities, and the simultaneous investigation of both glycolysis and autophagy pathways. However, several important limitations must be acknowledged. First, K562 is derived from chronic myeloid leukemia rather than AML; thus, our conclusions regarding AML biology are primarily based on HL-60 cells, and cross-comparisons between the two should be interpreted with caution. Second, all experiments were conducted under normoxic conditions (21% oxygen), which do not recapitulate the hypoxic bone marrow microenvironment where HIF-1α is physiologically stabilized. Third, our analysis was limited to mRNA expression; we did not measure HIF-1α protein stability, LDHA enzymatic activity, NAD^+ /NADH ratios, autophagic flux, or the functional metabolic consequences such as lactate production or oxygen consumption. Fourth, we did not assess immune-related parameters, despite the known role of lactate in immunosuppression. Fifth, detailed characterization of cell death pathways, including apoptosis, necrosis, ROS production, and cell cycle progression, was not performed. Finally, this in-vitro study lacks in-vivo validation, which limits the translational relevance of our findings. Future investigations should incorporate hypoxic culture conditions, functional metabolic assays, and animal models to definitively establish the therapeutic potential of targeting HIF-1α and LDHA in AML.

Conclusion

Our study demonstrates that HIF-1α and LDHA are associated with the transcriptional regulation of glycolysis- and autophagy-related genes in leukemia cells. Silibinin treatment is associated with changes in the expression of key glycolytic genes, including GLUT1 and PKM2, as well as autophagy-related genes in HL-60 and K562 cells. Sodium oxamate also induces heterogeneous transcriptional responses in glycolysis-related genes, suggesting adaptive regulation under metabolic perturbation. Importantly, these findings reflect transcriptional associations under in-vitro conditions rather than direct evidence of functional metabolic inhibition or therapeutic efficacy. Overall, targeting HIF-1α and LDHA may influence metabolic gene expression in leukemia cells; however, further mechanistic studies, including metabolic flux analysis and in-vivo validation, are required to determine their true therapeutic potential.

Data Availability

Data are available upon reasonable request from the corresponding author.

Acknowledgements
The authors would like to thank the staff of the Department of Immunology of Mazandaran University of Medical Sciences, Sari, Iran for their cooperation for providing laboratory environment.

Funding
This study was supported by Mazandaran University of Medical Sciences (Project number: 23787).

Authors' Contribution
M.S. and T.E. contributed to formal analysis, investigation, methodology, software, and writing—original draft. A.R. and A.K. contributed to conceptualization and investigation. R.V. and H.A-O contributed to methodology and conceptualization. M.T. and S.T. contributed to conceptualization, data curation, formal analysis, methodology, project administration, resources, software, supervision, validation, visualization, and writing—review and editing.
All authors have read and approved the final version, and agreed to be accountable for all aspects of the work and to ensure that questions regarding the accuracy or integrity of any part of the work are appropriately reviewed and resolved.

Conflict of Interest
None declared.

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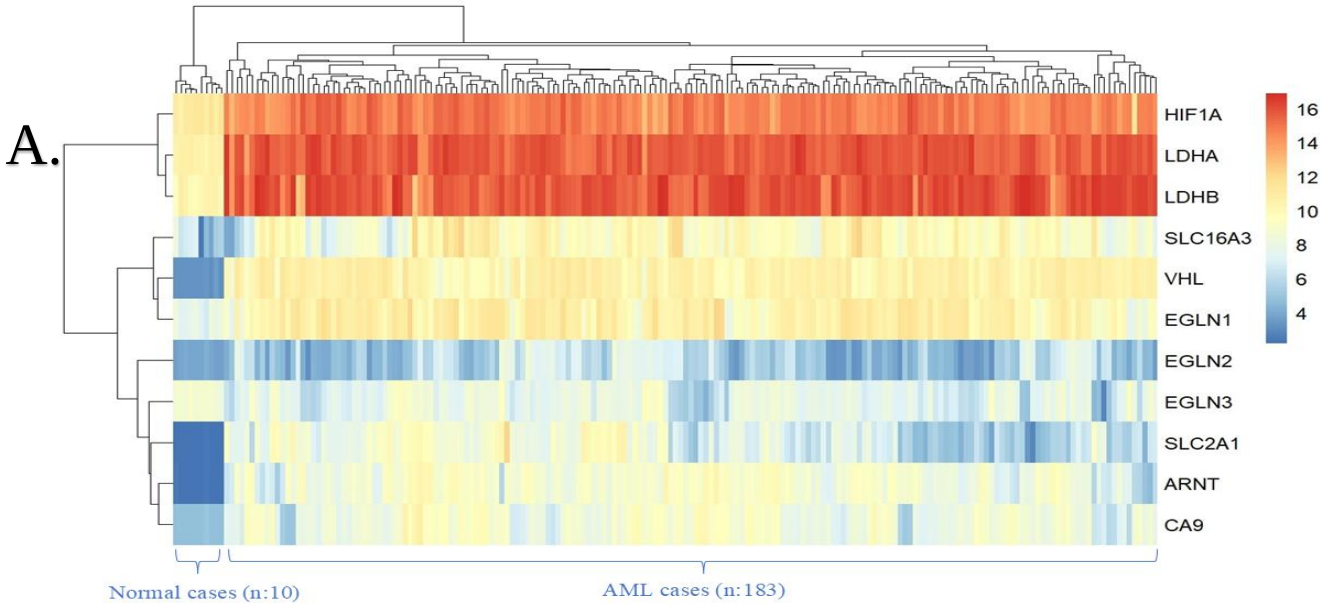
Table 1. Primer sequences used for qRT-PCR

Gene	Sequences	Product size
GLUT1	F: GGGTTGTGCCATACTCAT R: GAAGAGTTCAGCCACGAT	151 bp
HK2	F: CGGATGTGTATCAATATGGAGT R: CTTCTCAAACAGTTGCTTTCC	126 bp
PKM2	F: GGTGTTTGCGTCATTCATC R: GCCTCCAGGATTTCATCAA	141 bp
ATG7	F: AGGAGATTCAACCAGAGAC R: GAGGCTCATTCATCCGAT	170 bp
Beclin1	F: ATCAGGAGGAAGCTCAGTAT R: GGCATAACGCATCTGGTT	104 bp
ATG5	F: CACAAGCAACTCTGGATG R: CAGTCGTTGTCTGATATATTCTAA	136 bp
β-actin	F: CCTTCCTGGGCATGGAGTCCT R: TGGGTGCCAGGGCAGTGAT	174 bp

F: forward primer; R: reverse primer; qRT-PCR: Quantitative reverse-transcription polymerase chain reaction; bp: base pairs; GLUT1: Glucose transporter 1; HK2: Hexokinase 2; PKM2: Pyruvate kinase M2; ATG7: Autophagy-related 7; Beclin-1, BECN1: (coiled-coil, moesin-like BCL2-interacting protein); ATG5: Autophagy-related 5



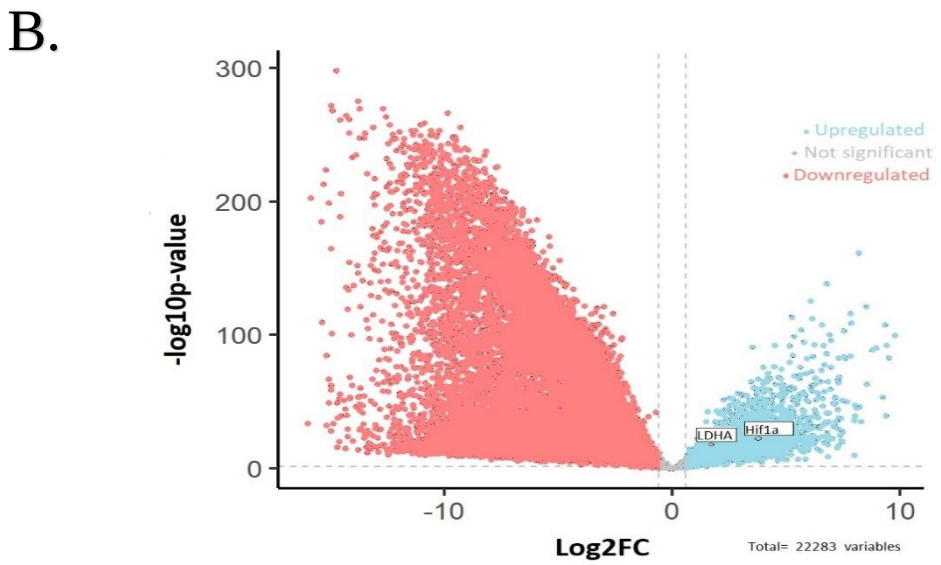


Figure 1. HIF-1α and LDHA are significantly upregulated in AML patients compared with normal controls. (A) Heatmap of HIF1A and LDHA expression in 183 AML samples and 10 normal bone marrow controls from GTEx. Red indicates high expression, blue low expression. (B) Volcano plot showing statistical significance (−log10 FDR) vs. fold change (log2). HIF1A and LDHA are highlighted in red (FDR<0.05, log2FC>1.5).

HIF-1α: Hypoxia-inducible factor 1-alpha; LDHA: Lactate dehydrogenase A; AML: Acute myeloid leukemia; GTEx: Genotype-Tissue Expression; log2FC: log2 fold change

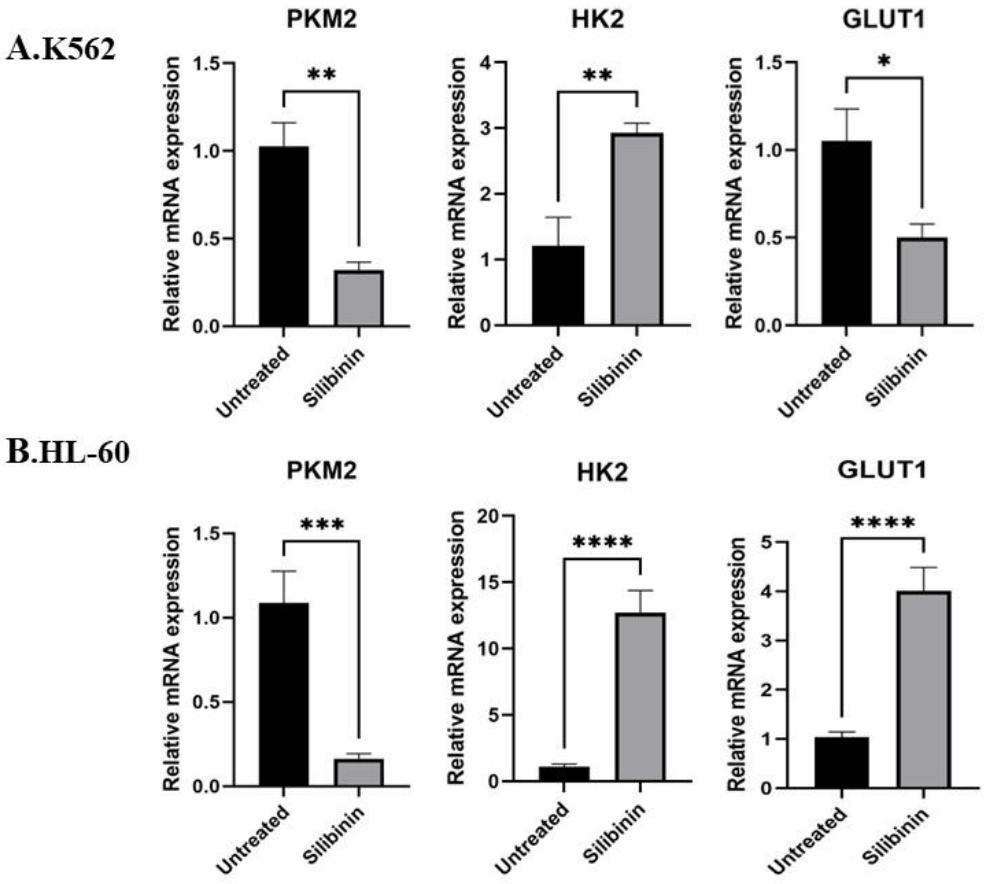


Figure 2. Silibinin-mediated HIF-1α inhibition suppresses glycolytic gene expression in K562 and HL-60. K562 (A) and HL-60 (B) cells were treated with Silibinin for 48 hours. Relative mRNA expression levels of GLUT1, HK2, and PKM2 were analyzed by qRT-PCR. Experiments were performed in duplicate (n = 6). Data are presented as mean ± SEM. Statistical significance was determined using an unpaired t-test.

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. GLUT1: Glucose transporter 1; HK2: Hexokinase 2; PKM2: Pyruvate kinase M2; qRT-PCR: quantitative reverse-transcription polymerase chain reaction; SEM: Standard error of the mean

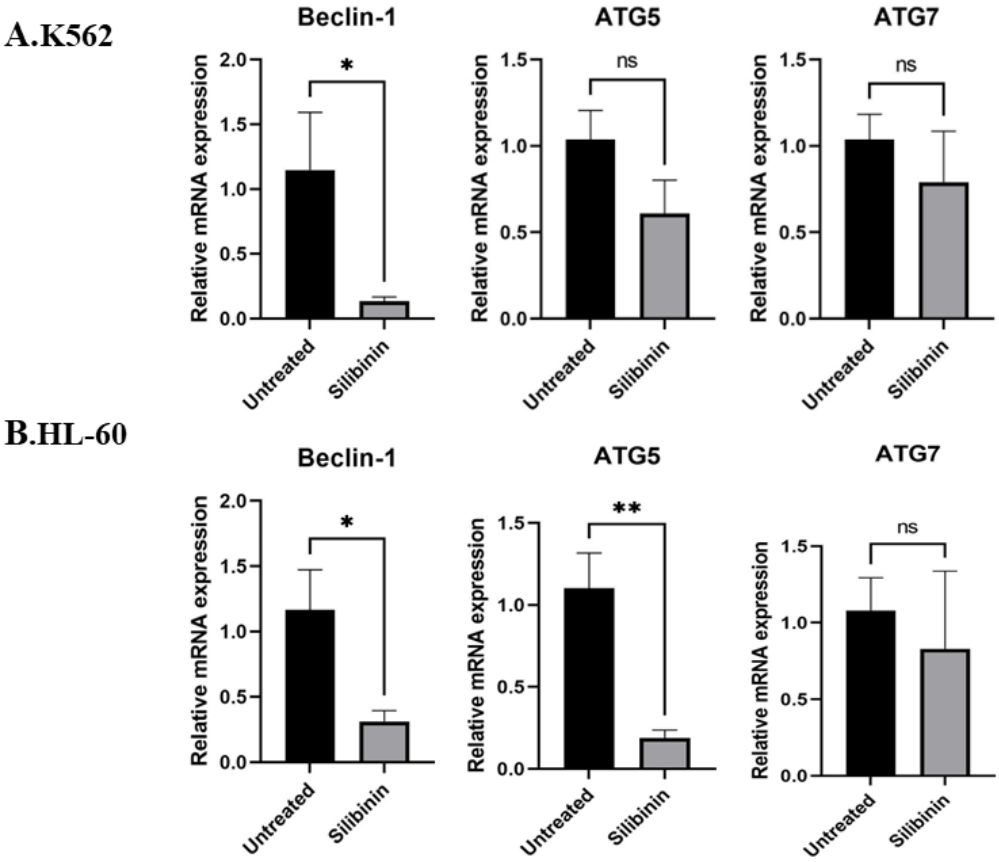


Figure 3. Effect of Silibinin on autophagy-related gene expression in K562 and HL-60. K562 (A) and HL-60 (B) cells were treated with Silibinin for 48 hours. Relative mRNA expression levels of ATG5, Beclin-1, and ATG7 were analyzed by qRT-PCR. Experiments were performed in duplicate (n = 6). Data are presented as mean ± SEM. Statistical significance was determined using an unpaired t-test.

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. ATG5: Autophagy-related 5; Beclin-1, BECN1: (coiled-coil, moesin-like BCL2-interacting protein); ATG7: Autophagy-related 7; qRT-PCR: quantitative reverse-transcription polymerase chain reaction; SEM: Standard error of the mean

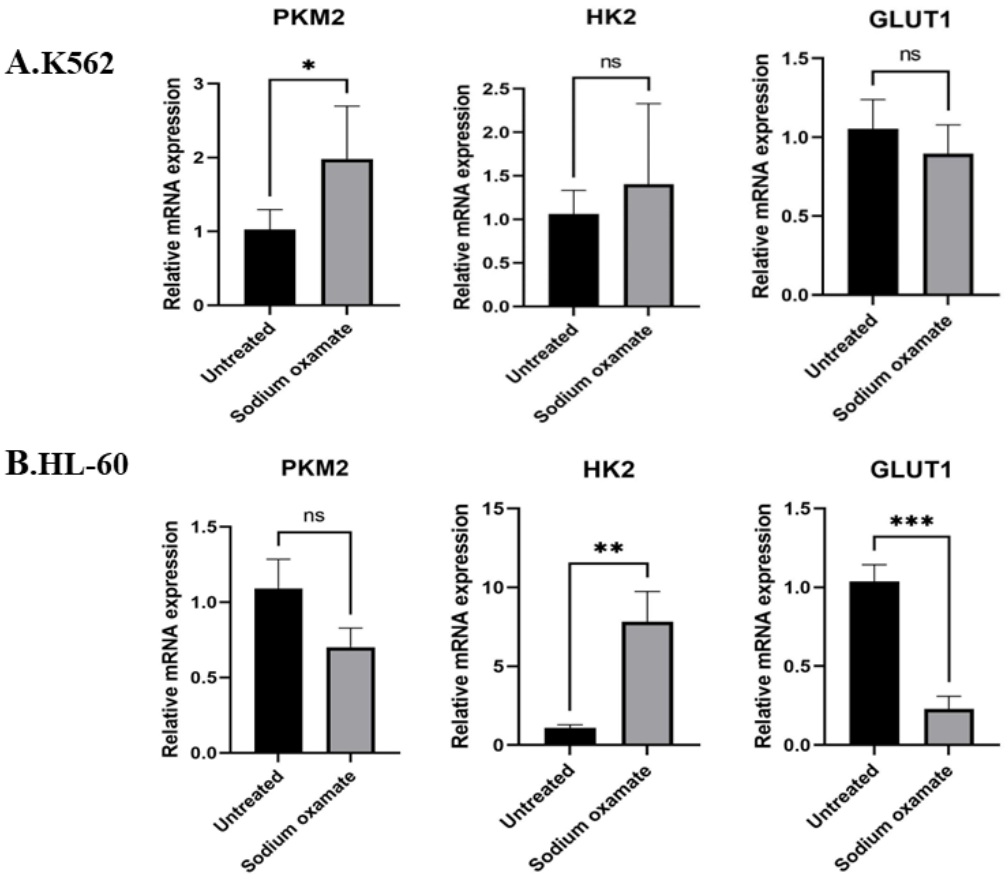


Figure 4. Effect of Sodium Oxamate on glycolytic gene expression in K562 and HL-60. K562 (A) and HL-60 (B) cells were treated with Sodium Oxamate for 48 hours. Relative mRNA expression levels of GLUT1, HK2, and PKM2 were analyzed by qRT-PCR. Experiments were performed in duplicate (n = 6). Data are presented as mean ± SEM. Statistical significance was determined using an unpaired t-test.

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. GLUT1: Glucose transporter 1; HK2: Hexokinase 2; PKM2: Pyruvate kinase M2; qRT-PCR: quantitative reverse-transcription polymerase chain reaction; SEM: Standard error of the mean

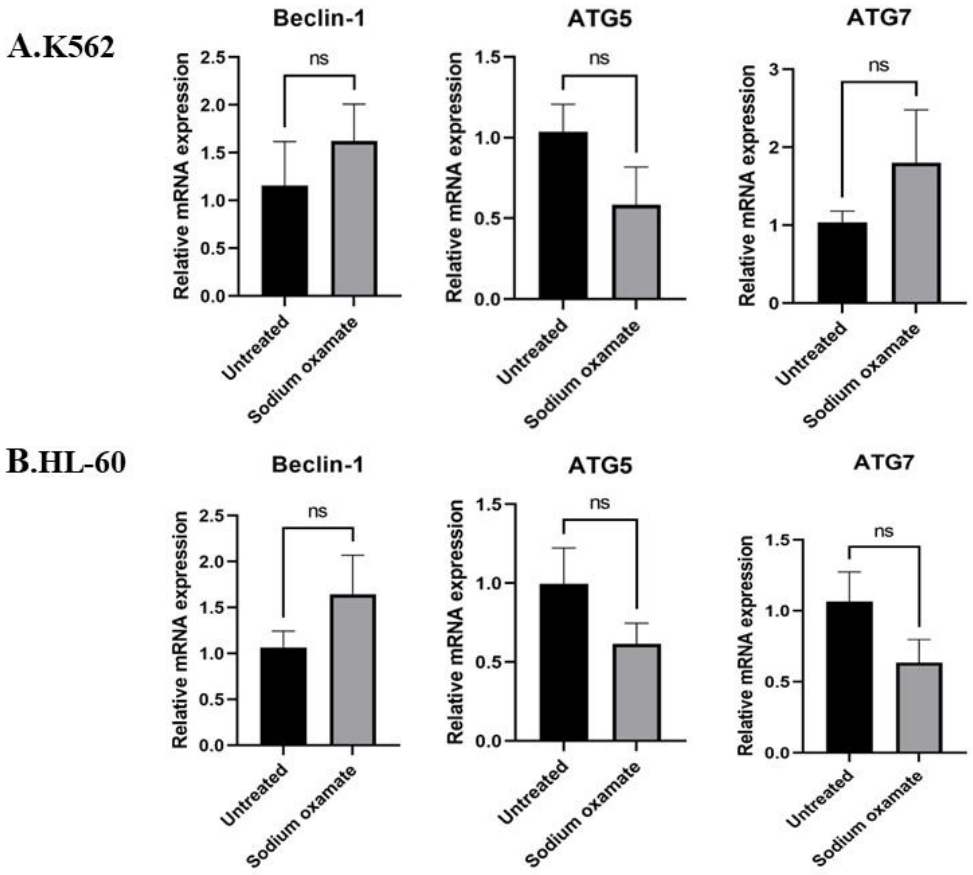


Figure 5. This figure shows the effect of Sodium Oxamate on autophagy-related gene expression in K562 and HL-60. K562 (A) and HL-60 (B) cells were treated with Sodium Oxamate for 48 hours. Relative mRNA expression levels of ATG5, Beclin-1, and ATG7 were measured by qRT-PCR. Experiments were performed in duplicate (n = 6). Data are presented as mean ± SEM. Statistical significance was determined using an unpaired t-test.

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. ATG5: Autophagy-related 5; Beclin-1, BECN1: (coiled-coil, moesin-like BCL2-interacting protein); ATG7: Autophagy-related 7; qRT-PCR: quantitative reverse-transcription polymerase chain reaction; SEM: Standard error of the mean