



Ganoderic Acid A Extract Induces Apoptosis and Upregulates Autophagy-Related Genes in NALM-6 Cell Line: A Potential Therapeutic Agent for Acute Lymphoblastic Leukemia

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is the most common leukemia in children and is associated with a high relapse rate despite current treatments. Ganoderic acid A (GAA) is a bioactive compound found in *Ganoderma lucidum* that has shown potential antileukemic properties.

Objectives: This study aimed to investigate the effect of a GAA extract on autophagy gene expression and apoptosis induction in the NALM-6 cell line.

Methods: A total of 20×10^3 NALM-6 cells were cultured in triplicate across various conditions: Culture condition supplemented with GAA, culture condition supplemented with L-asparaginase, culture condition supplemented with both GAA and L-asparaginase, and culture condition alone without L-asparaginase and GAA. The optimal concentration of GAA treatment was determined using an MTT assay. Flow cytometry was used to assess cell death induced by GAA treatment, using FITC-conjugated propidium iodide (PI) and annexin V staining. The expression levels of autophagy-related genes, including MAP1LC3B, BECN1, ATG5, ATG10, RB1CC1, and AMBRA1, were measured in the groups using real-time polymerase chain reaction.

Results: The results of the MTT test indicated that the half maximum inhibitory concentration (IC_{50}) of GAA against leukemic cells was 140 $\mu\text{g/mL}$ after 48 hours of treatment. Moreover, flow cytometry analysis demonstrated a 40.5% increase in apoptosis and cell death at a concentration of 140 $\mu\text{g/mL}$ of GAA after 48 hours. Furthermore, treatment with GAA resulted in upregulation of the expression of MAP1LC3B ($P = 0.024$), BECN1 ($P = 0.035$), ATG5 ($P = 0.024$), ATG10 ($P = 0.024$), RB1CC1 ($P = 0.024$), and AMBRA1 ($P = 0.024$) in NALM-6 cells compared to the control groups.

Conclusions: These findings suggest that GAA has potential as a therapeutic agent for ALL. The GAA might have potential therapeutic properties against ALL.

Keywords: Acute Lymphoblastic Leukemia, Ganoderic Acid A, Autophagy, Apoptosis, NALM-6 Cell Line, Therapeutic Agent, Flow Cytometry

1. Background

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. While L-asparaginase induces complete remission in over 85% of patients,

approximately half of the cases relapse with unfavorable outcomes (1). Current therapies have adverse side effects that decrease life expectancy, highlighting the need to reduce cytotoxic effects and explore better treatment regimens (2-4). The Reishi or

Lingzhi mushroom is widely used in China and other Asian countries for its pharmacological activity, primarily due to its polysaccharides and triterpenoids (5). Ganoderic Acid A (GAA), isolated from *Ganoderma lucidum*, exhibits remarkable cytotoxic effects against various cancers. It suppresses cell growth, promotes apoptosis, and can serve as an additional therapeutic intervention for malignancies (6-8).

Autophagy is a crucial cellular pathway that maintains homeostasis and can help cells survive during stress. However, autophagy induction can increase tumor cell survival under adverse conditions, potentially playing a role in leukemia development (9). This study investigated GAA's antineoplastic effects on NALM-6 cells, focusing on changes in autophagy gene expression (MAP1LC3B, BECN1, ATG5, ATG10, RB1CC1, and AMBRA1) before and after treatment with GAA extract of *G. lucidum* and its relationship with apoptosis induction (10).

2. Objectives

The study aims to provide insight into GAA's potential as a natural antineoplastic agent for ALL treatments, with fewer side effects.

3. Methods

3.1. Study Design

In this case-control study, the NALM-6 B-cell line was obtained from the Iranian Biological Resource Center (IBRC) and cultured in RPMI-1640 media supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 100 U/mL penicillin-streptomycin solution. All ingredients and reagents used in cell culture were provided by Sigma-Aldrich (Munich, Germany). Cell viability was ensured using conventional methods and the trypan blue exclusion technique. The GAA extract, purchased from Sigma-Aldrich (St. Louis, MO, USA), was stored at -80°C in a dimethyl sulfoxide (DMSO) stock solution at a concentration of 5 mg/mL.

3.2. MTT Assay

To assess the inhibitory effect of GAA on cell metabolic activity, an MTT assay was conducted. NALM-6 cells were seeded in a 96-well plate and cultured for 24, 48, and 72 hours with different concentrations of GAA (25, 50, 100, 200, and 400 µg/mL). A total of 20 µL of freshly prepared MTT solution was added to each well, and the plate was incubated before measuring the spectrometric absorbance at 570 nm using a BioTek ELx800 microplate photometer (BioTek, Winooski, VT,

USA). The IC₅₀, the concentration of medication that inhibited cell growth by 50%, was calculated. Each experiment was performed in triplicate.

3.3. RNA Extraction and Complementary DNA Synthesis

Cellular RNA was extracted from each group using trizol reagent (Thermo Fisher Scientific, USA). The amount and quality of RNA were measured using an RNA spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, USA), and the RNA was then stored at -80°C. Next, 3.7 µL of each RNA sample was reverse-transcribed into complementary DNA (cDNA) using prime script first strand cDNA synthesis kit (Pars Toos, Tehran, Iran) following the manufacturer's instructions, bringing the final volume to 20 µL.

3.4. Quantitative Real-time PCR

Primers for MAP1LC3B, ATG5, ATG10, RB1CC1, and AMBRA1 were designed using AlleleID version 7 and Gene Runner software (Table 1). Quantitative real-time PCR (qRT-PCR) was performed using a QIAGEN real-time PCR cycler (Rotor-Gene, Germany), with ACTB (beta-actin) as the endogenous control to evaluate gene expression.

Each real-time PCR reaction contained cDNA, SYBR Green PCR master mix (SYBR premix Ex Taq II, Tli RNaseH Plus, Yektatajhz, Iran), primers, and nuclease-free water in a final volume of 20 µL, and was run for 45 cycles under specific conditions. The qRT-PCR assay was conducted under the following conditions: Initial denaturation at 95°C for five minutes, followed by 45 cycles of 95°C for five seconds, 59°C for 20 seconds, and 72°C for 30 seconds. Each sample was analyzed in triplicate. The $2^{-\Delta\Delta CT}$ method was used to analyze fold changes (FC) in gene expression, with each sample run in triplicate. The comparative Ct method ($RQ = 2^{-\Delta\Delta CT}$) was used to determine the relative expression levels of all genes.

3.5. Flow Cytometry

The percentage of apoptotic cells following GAA treatment was measured using a kit developed by BD Biosciences (San Jose, CA). Briefly, NALM-6 cells were collected 48 hours after treatment with the specified dose of GAA isolated from *G. lucidum*. The cells were washed twice with phosphate-buffered saline (PBS) before resuspending 5×10^5 cells in 1 mL of 1X annexin V binding buffer. The cells were then incubated in the dark for 20 minutes at room temperature with 5 µL of fluorochrome-conjugated annexin V and 5 µL of propidium iodide (PI) staining solution. After

Table 1. Primer Sequences for Real-time RT-PCR

Genes	Primer Pair	Product Length (bp)	Product Melt Temperature (°C)
ACT-β		177	90
Forward	ATCGTGCCTGACATTAAGGAG		
Reverse	GAAGGAAGGCTGGAAGAGTG		
MAP1LC3		208	85
Forward	GTGATAATAGAACGATACAAGG		
Reverse	CACTCTCATAACCTCTG		
BECN1		154	87
Forward	TGGCACAATCAATAACTTC		
Reverse	GTAAGGAACAAGTCGGTAT		
ATG5		152	83
Forward	TTGCTCTCTGCTATTGAT		
Reverse	GCTGTGGGATGATACTAAT		
ATG10		185	84
Forward	TGATTGTGAAGTGATTGAAAC		
Reverse	GTAGCAGTCGCATCTTAT		
RB1CC1		99	80
Forward	TGTCGTCTCCTAATCCTAT		
Reverse	GCATTGCTACTGTCTAT		
AMBRA1		93	82
Forward	CGCTCTACCTTCTTATTGG		
Reverse	AGTCTTCACCTCCGTAATA		

incubation, 400 µL of binding buffer was added to the cell suspension, which was then centrifuged at 400 g for 5 minutes. Cells that were annexin V-positive/PI-negative were considered to be in the early stages of apoptosis, while those that were positive for both annexin V and PI were believed to be in the late stages of apoptosis or necrosis. A FACSCalibur flow cytometer (BD Biosciences, USA) was used to identify the apoptotic population, and the data were analyzed using FlowJo software (TreeStar LLC, USA).

3.6. Statistical Analysis

The results are presented as the median FC in expression from three separate studies. GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA) was used for analysis. One-way ANOVA and Fisher's exact test were conducted to compare different groups, with a significance level set at $P < 0.05$.

4. Results

4.1. The Significance of the Anti-proliferative Effects of Ganoderic Acid A on Human NALM-6 Cancer Cells

The present study found that GAA significantly reduced the proliferation of NALM-6 cells. An MTT assay was performed to assess cell viability after treatment

with different concentrations of GAA (25, 50, 100, 200, and 400 µg/mL) for 24, 48, and 72 hours. The results demonstrated a dose- and time-dependent decrease in cellular viability. The IC_{50} value was determined to identify the optimal GAA concentration, and the NALM-6 cells exhibited significantly reduced viability in the presence of 140 µg/mL of GAA for 48 hours ($P < 0.0001$). These findings clearly demonstrate the antiproliferative effects of GAA on human NALM-6 cancer cells (Figure 1). Notably, subsequent tests were conducted using 140 µg/mL of GAA after 48 hours of treatment.

4.2. Effect of Ganoderic Acid A Extract on Apoptosis in NALM-6 Cells

Based on the results presented in Figure 2, the negative control group of NALM-6 cells (without any treatment) had a baseline apoptosis rate of 10.15%. Figure 2B shows NALM-6 cells treated with DMSO to assess any potential side effects of DMSO on NALM-6 cells, and this group had a similar apoptosis rate of 9.13% compared to the negative control group (Figure 2A). Figure 2C shows NALM-6 cells treated with L-asparaginase as the positive control, which had a significantly higher apoptosis rate of 33.4% compared to the negative control and DMSO-treated cells ($P < 0.001$; Figure 2). In Figure 2D, the NALM-6 cells were treated

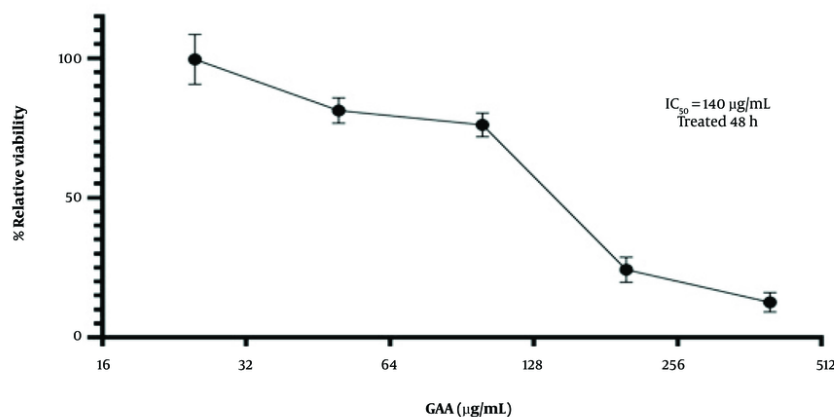


Figure 1. The IC₅₀ of ganoderic acid A (GAA) treatment in the Nalm-6 cells was determined by MTT assay. The Nalm-6 cells (2×10^4 cells/well) were plated into 96-well plates and treated with increasing concentrations (25, 50, 100, 200, and 400 µg/mL) of GAA for 24, 48 and 72 hours. Data are shown as mean \pm SD in triplicate. The cells treated without GAA were used as the controls.

with GAA at a concentration of 140 µg/mL for 48 hours to evaluate the induction of apoptosis. The findings demonstrated a significantly higher proportion of apoptotic cells in the treated cells compared to the control groups. Specifically, our results indicated 40.5% of early and late apoptosis after 48 hours of treatment ($P < 0.0021$; Figure 2).

4.3. Comparison of Autophagic Gene Expression in NALM-6 Cells Treated with Ganoderic Acid A Versus L-Asparaginase

This study reports the first evaluation of GAA's effect on autophagy-related gene expression in NALM-6 cells using qRT-PCR. In Figure 3, we display the effect of 140 µg/mL GAA treatment for 48 hours on the expression of six autophagy-related genes (BECN1, ATG5, ATG10, RB1CC1, AMBRA1, MAP1LC3B) in NALM-6 cells. Our results demonstrate that GAA treatment significantly increased the expression levels of these genes compared to untreated cells. Specifically, MAP1LC3B ($P = 0.024$; median FC of expression = 111), BECN1 ($P = 0.035$; FC = 46.2), ATG5 ($P = 0.024$; FC = 165), ATG10 ($P = 0.024$; FC = 80.44), RB1CC1 ($P = 0.024$; FC = 1184), and AMBRA1 ($P = 0.024$; FC = 67.64) were significantly upregulated (Table 2). Figure 4 illustrates these findings with a heat map.

5. Discussion

Ganoderic acid A is a triterpenoid from *G. lucidum* used in Chinese herbal medicine to treat various diseases, including cancer (11). It has significant anticancer properties against various human cancer

cells in vitro, but its molecular mechanism in NALM-6 B-cell leukemia is unknown (12). Apoptosis is a cell death process that can prevent the proliferation of malignant tumors (13, 14). Previous studies have examined the effect of *G. lucidum* on apoptosis in ALL cell lines, and in this study, GAA was selected as a potential inhibitor of leukemic cell proliferation (15, 16).

Results showed that GAA induced apoptosis in NALM-6 cells, with a rate of 40.3% compared to 33.4% in L-asparaginase-treated cells (Figure 2C). This suggests that GAA has the potential to reduce cell proliferation in NALM-6 cells by inducing apoptosis, making it a promising natural cancer treatment. Many investigations have shown that autophagy has a dual impact on cancer, either inhibiting tumor growth or halting cancer advancement, which appears to rely on unidentified features of diverse cancer forms. This analysis outlines the main objectives related to autophagy and the evolution of malignancy. Furthermore, the conflicting roles of autophagy in cancer as a suppressor of tumors or as a promoter of cancer, along with potential treatment options using modulators of autophagy or a combination of therapies with anticancer medications, have been explored (17).

Autophagy plays a role in leukemia initiation and progression, as leukemic cells use it for metabolic demands for proliferation and survival (Figure 5). Activating autophagy can also promote leukemogenesis and drug resistance, making it a potential therapeutic target for leukemia treatment (Figure 4) (18, 19).

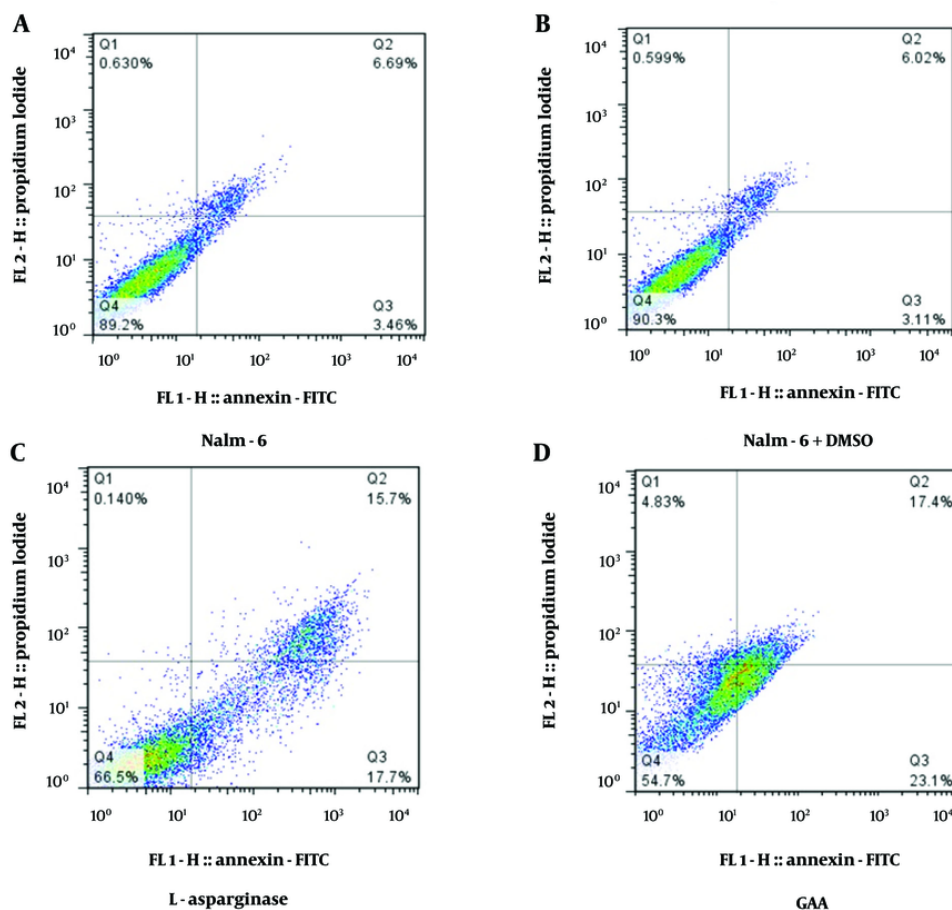


Figure 2. The effects of Ganoderic acid A (GAA) and L-asparaginase on apoptosis in Nalm-6 cells were compared to two control groups: Nalm-6 cells and Nalm-6 cells treated with DMSO. A, Flow cytometry chart showing the distribution of necrotic cells (Q1), late apoptosis (Q2), early apoptosis (Q3), and live cells (Q4); B, the effect of DMSO as a control on Nalm-6 cells; C, results from the induction of apoptosis by L-asparaginase on the Nalm-6 cell line. The cells were exposed to 8 μ M of L-asparaginase, and the induction of apoptosis after 48 hours of incubation was compared with untreated cells ($P = 0.0112$); D, results from the induction of apoptosis by treatment with 140 μ g/mL of GAA on the Nalm-6 cell line. The Nalm-6 cells were exposed to the intended concentration of the GAA extract, and the induction of apoptosis after 48 hours of incubation was evaluated ($P = 0.0021$). The error bars in the diagram represent the standard deviations of two separate tests.

The current study found that GAA treatment decreased cell proliferation, induced apoptosis and autophagy, and was cytotoxic to NALM-6 cells in vitro. These results are consistent with previous studies that demonstrated the cytotoxic effects of GAA on various cancer cell lines, including leukemia (20). These findings suggest that GAA may have potential as a natural agent for inhibiting leukemic cell proliferation, promoting apoptosis and autophagy, and developing novel therapeutic strategies for leukemia.

In this study, we used qRT-PCR to assess the expression of autophagic genes, including MAP1LC3B, BECN1, ATG5, ATG10, RB1CC1, and AMBRA1, in NALM-6 cells

before and after treatment with GAA extract, as shown in Figure 3. Our results showed that GAA treatment significantly increased the expression levels of these autophagic genes compared to the control groups, which included untreated cells and cells treated with L-asparaginase (Figure 3). These findings are consistent with previous studies by Reis et al., who studied breast cancer cells and found that *G. lucidum* induced autophagy by increasing the expression of ULK1, Atg5, Atg12, Beclin1, and LC3, which reduced cell growth. Additionally, Li et al. conducted a study on colorectal cancer cells, including SW620 cells, and demonstrated that *G. lucidum* reduced cancer cell growth, decreased Bcl-2 expression, increased apoptosis, and induced

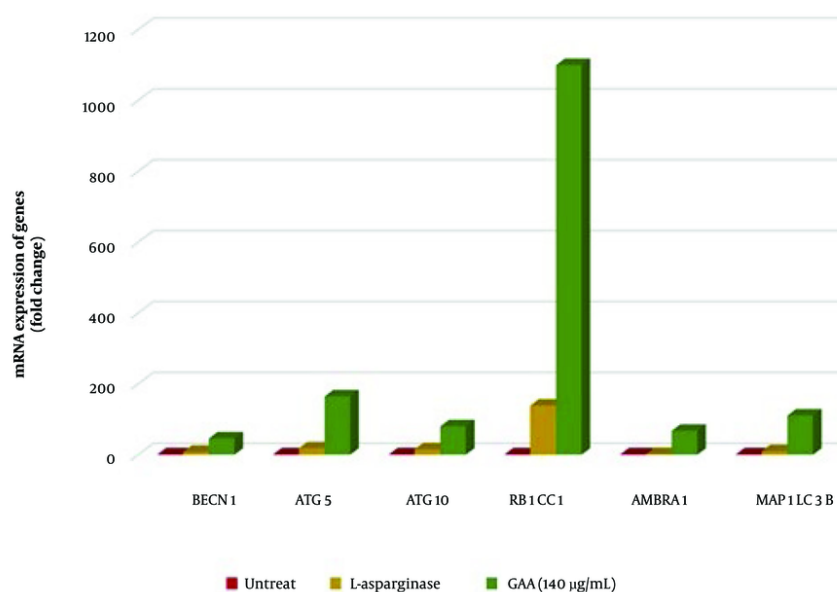


Figure 3. Quantitative real-time PCR (qRT-PCR) analysis revealed significant differences in the expression MAP1LC3B, BECN1, ATG5, ATG10, RB1CC1, and AMBRA1 genes in Nalm6 cells treated with Ganoderic acid A (GAA) and L-ASP compared to untreated cells. All genes showed upregulation ($P < 0.0001$) in response to GAA treatment compared to untreated cells. Similarly, the expression levels of all genes were increased in response to L-ASP treatment compared to untreated cells.

Table 2. Fold Change of the Following Genes After Treatment with Ganoderic Acid A and L-asparaginase

Genes	Treatment with	FC
Becline	GAA	46.2
	L-asparaginase	8.33
ATG5	GAA	165
	L-asparaginase	18.37
ATG10	GAA	80.44
	L-asparaginase	15.13
Fib200	GAA	1184
	L-asparaginase	139
AMBRA	GAA	67.64
	L-asparaginase	2.158
LC3	GAA	111
	L-asparaginase	10.26

Abbreviations: GAA, Ganoderic acid A; FC, fold change.

autophagy (18, 21-23). Furthermore, Reis et al. investigated the effect of methanolic extract of *G. lucidum* on gastric adenocarcinoma cancer cells and found that it increased LC3-II levels, decreased P62 levels, and induced autophagy (21). These studies, along with our findings, suggest that *G. lucidum* and its

components, such as GAA, have the potential to induce autophagy and reduce cell growth.

In a study on acute myeloid leukemia patients, it was found that the expression of the *BECN1* gene was higher in AML patients than in controls, and low expression may be associated with complete recovery. This difference may be due to the dual role of autophagy

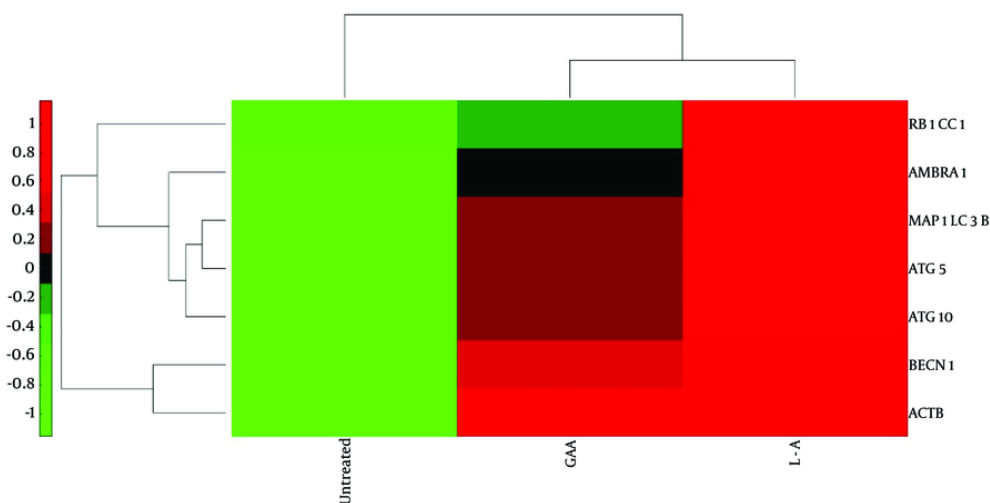


Figure 4. Heat map was generated using hierarchical clustering to visualize the expression MAPI3C3B, BECN1, ATG5, ATG10, RBIC1, and AMBRA1 in Nalm6 cells treated with Ganoderic acid A (GAA), L-ASP, and untreated cells. The red color indicates lower expression levels compared to the reference channel, while the green color indicates higher expression levels. The heat map showed that GAA treatment resulted in increased expression levels of all six genes, as indicated by the abundance of green-colored cells. In contrast, untreated cells and those treated with L-ASP showed lower expression levels of these genes, as indicated by the abundance of red-colored cells.

in tumorigenesis and the pathogenesis of ALL, as well as differences in autophagic activity between populations and ALL subgroups (21). Surprisingly, no previous study has investigated the effect of GAA on the expression of autophagy genes in leukemia cells. This study is the first to evaluate this effect, showing that GAA treatment increased the expression levels of autophagy genes in NALM-6 cells, which may contribute to the therapeutic effect of GAA and increase cell death.

The PI3K/AKT/mTOR pathway regulates many biological processes, including cell proliferation, differentiation, survival, apoptosis, autophagy, tumorigenesis, and angiogenesis. The GAA has been shown to strongly inhibit this pathway in human glioblastoma cells in previous studies (23). The PI3K/AKT/mTOR signaling network plays a role in the occurrence and progression of tumors, including leukemia (24). Inactivation of at least one component of this pathway has been associated with tumorigenesis. mTOR is a crucial regulator of autophagy and inhibits this pathway. Additionally, some signaling pathways, including the class I PI3K/Akt pathway, can inhibit both autophagy and apoptosis pathways. Autophagy can either promote cell survival by inhibiting apoptosis or activate apoptosis, making it an antagonist of apoptosis. Targeting the PI3K/AKT/mTOR pathway may be a potential therapeutic strategy for cancer treatment, including leukemia (25).

The GAA treatment inhibits this pathway, contributing to its antitumor effects, including apoptosis and autophagy induction, and may have the potential for developing cancer therapeutics. The GAA is hypothesized to increase the expression of autophagy genes, particularly RBIC1, and inactivate the PI3K/AKT/mTOR signaling pathway, leading to apoptosis in NALM-6 cells. Ganoderic acid A has the potential to inhibit cancer cell growth and expansion and increase the expression of autophagy genes. However, more research is needed to determine the exact mechanisms of GAA as an anticancer agent for leukemia treatment (26).

The use of GAA or other active compounds derived from medicinal plants, alone or in combination with chemotherapy drugs, may offer a promising approach for achieving better treatment outcomes with fewer side effects, particularly for ALL, a common and fatal disease among children. In conclusion, the study found that GAA has a potent cytotoxic effect on NALM-6 cells by inducing apoptosis and increasing the expression levels of autophagic genes, including MAPI3C3B, BECN1, ATG5, ATG10, RBIC1, and AMBRA1. These results suggest that GAA may be a natural agent for the treatment of acute B lymphoblastic leukemia with potentially fewer side effects. Our study sheds new light on the potential mechanisms underlying GAA's therapeutic effects in leukemia.

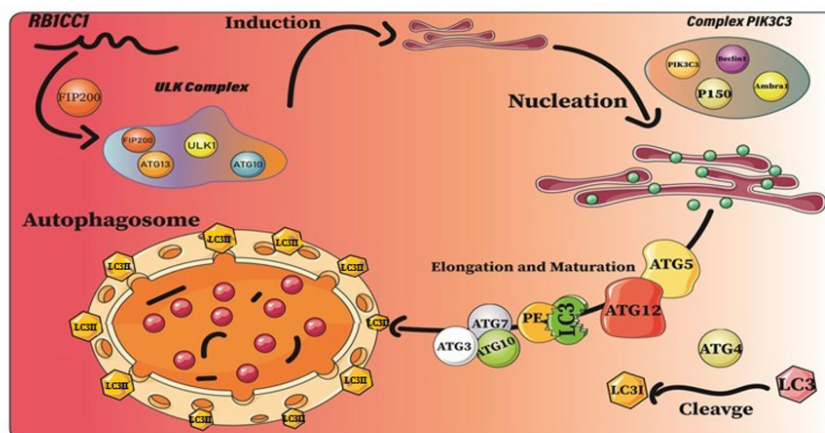


Figure 5. Mechanism of autophagy. The mechanism of autophagy has a dichotomous role in cancer cells, as it can either promote or inhibit cell proliferation depending on the context. While autophagy can sometimes provoke cancer formation, it can also cause inhibition of proliferation.

Footnotes

Authors' Contribution: Study concept and design: T. Gh.; Analysis and interpretation of data: N. N. and T. Gh.; Drafting of the manuscript: T. S. and B. A.; Critical revision of the manuscript for important intellectual content: T. Gh., and N. N.; Statistical analysis: M. F.; Acquisition of data: Sh. H. and F. R.; Administrative, technical, and material support: T. Gh. and N. N.

Conflict of Interests Statement: The authors declared no conflict of interests.

Data Availability: No new data were created or analyzed in this study. Data sharing does not apply to this article.

Ethical Approval: This study was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1400.053).

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References

- Zou Y, Mei D, Yuan J, Han J, Xu J, Sun N, et al. Preparation, Characterization, Pharmacokinetic, and Therapeutic Potential of Novel 6-Mercaptopurine-Loaded Oral Nanomedicines for Acute Lymphoblastic Leukemia. *Int J Nanomedicine*. 2021;**16**:1127-41. [PubMed ID: 33603372]. [PubMed Central ID: PMC7886780]. <https://doi.org/10.2147/IJN.S290466>.
- Bhojwani D, Pui CH. Relapsed childhood acute lymphoblastic leukaemia. *Lancet Oncol*. 2013;**14**(6):e205-17. [PubMed ID: 23639321]. [https://doi.org/10.1016/S1470-2045\(12\)70580-6](https://doi.org/10.1016/S1470-2045(12)70580-6).
- Pui CH. Precision medicine in acute lymphoblastic leukemia. *Front Med*. 2020;**14**(6):689-700. [PubMed ID: 33074527]. [PubMed Central ID: PMC9671279]. <https://doi.org/10.1007/s11684-020-0759-8>.
- Zhao HL, Cui SY, Qin Y, Liu YT, Cui XY, Hu X, et al. Prophylactic effects of sporoderm-removed *Ganoderma lucidum* spores in a rat model of streptozotocin-induced sporadic Alzheimer's disease. *J Ethnopharmacol*. 2021;**269**:113725. [PubMed ID: 33352241]. <https://doi.org/10.1016/j.jep.2020.113725>.
- Li C, Cui Y, Lu J, Meng L, Ma C, Liu Z, et al. Spectrum-effect relationship of immunologic activity of *Ganoderma lucidum* by UPLC-MS/MS and component knock-out method. *Food Sci Hum Wellness*. 2021;**10**(3):278-88. <https://doi.org/10.1016/j.fshw.2021.02.019>.
- Tian YZ, Wang ZF, Liu YD, Zhang GZ, Li G. The whole-genome sequencing and analysis of a *Ganoderma lucidum* strain provide insights into the genetic basis of its high triterpene content. *Genomics*. 2021;**113**(1 Pt 2):840-9. [PubMed ID: 33091546]. <https://doi.org/10.1016/j.ygeno.2020.10.015>.
- Cao F, Feng L, Ye L, Wang L, Xiao B, Tao X, et al. Ganoderic Acid A Metabolites and Their Metabolic Kinetics. *Frontiers Pharmacol*. 2017;**8**. <https://doi.org/10.3389/fphar.2017.00101>.
- Ahmad MF, Wahab S, Ahmad FA, Ashraf SA, Abullais SS, Saad HH. *Ganoderma lucidum*: A potential pleiotropic approach of ganoderic acids in health reinforcement and factors influencing their production. *Fungal Biol Rev*. 2022;**39**:100-25. <https://doi.org/10.1016/j.fbr.2021.12.003>.
- Parzych KR, Klionsky DJ. An overview of autophagy: morphology, mechanism, and regulation. *Antioxid Redox Signal*. 2014;**20**(3):460-73. [PubMed ID: 23725295]. [PubMed Central ID: PMC3894687]. <https://doi.org/10.1089/ars.2013.5371>.
- Auberger P, Puissant A. Autophagy, a key mechanism of oncogenesis and resistance in leukemia. *Blood*. 2017;**129**(5):547-52. [PubMed ID: 27811111].

- 27956388]. <https://doi.org/10.1182/blood-2016-07-692707>.
11. Tamaddon G. Apoptosis Induction of Ganoderic Acid-A by Downregulating miR-125b and miR-365a-3p in Nalm-6 cells. *Haematol Int J*. 2022;**6**(2):1-10. <https://doi.org/10.23880/hij-16000201>.
 12. Shao J, Li Z, Jiao G, Sun G, Zhou Z. [Ganoderic acid A suppresses proliferation and invasion and induces apoptosis in human osteosarcoma cells]. *Nan Fang Yi Ke Da Xue Xue Bao*. 2015;**35**(5):619-24. ZH. [PubMed ID: 26018252].
 13. Zhang X, Xiao C, Liu H. Ganoderic Acid A Protects Rat H9c2 Cardiomyocytes from Hypoxia-Induced Injury via Up-Regulating miR-182-5p. *Cell Physiol Biochem*. 2018;**50**(6):2086-96. [PubMed ID: 30415259]. <https://doi.org/10.1159/000495053>.
 14. Vafadar A, Mokaram P, Erfani M, Yousefi Z, Farhadi A, Elham Shirazi T, et al. The effect of decitabine on the expression and methylation of the PPP1CA, BTG2, and PTEN in association with changes in miR-125b, miR-17, and miR-181b in NALM6 cell line. *J Cell Biochem*. 2019;**120**(8):13156-67. [PubMed ID: 30912184]. <https://doi.org/10.1002/jcb.28590>.
 15. Mortazavie F, Taheri S, Tandel P, Zare F, Tamaddon G. The effect of Ganoderic Acid A on miR-17-5p and miR-181b expression level and apoptosis induction in human leukemia Nalm-6 cells. *Iran J Pediatric Hematol Oncol*. 2022;**12**(3). <https://doi.org/10.18502/ijpho.v12i3.10058>.
 16. Wang X, Sun D, Tai J, Wang L. Ganoderic acid A inhibits proliferation and invasion, and promotes apoptosis in human hepatocellular carcinoma cells. *Mol Med Rep*. 2017;**16**(4):3894-900. [PubMed ID: 28731159]. [PubMed Central ID: PMC5646967]. <https://doi.org/10.3892/mmr.2017.7048>.
 17. Shin DW. Dual Roles of Autophagy and Their Potential Drugs for Improving Cancer Therapeutics. *Biomol Ther (Seoul)*. 2020;**28**(6):503-11. [PubMed ID: 33077698]. [PubMed Central ID: PMC7585634]. <https://doi.org/10.4062/biomolther.2020.155>.
 18. Li P, Liu L, Huang S, Zhang Y, Xu J, Zhang Z. Anti-cancer Effects of a Neutral Triterpene Fraction from *Ganoderma lucidum* and its Active Constituents on SW620 Human Colorectal Cancer Cells. *Anticancer Agents Med Chem*. 2020;**20**(2):237-44. [PubMed ID: 31749435]. <https://doi.org/10.2174/1871520619666191015102442>.
 19. Radwan FF, Hossain A, God JM, Leaphart N, Elvington M, Nagarkatti M, et al. Reduction of myeloid-derived suppressor cells and lymphoma growth by a natural triterpenoid. *J Cell Biochem*. 2015;**116**(1):102-14. [PubMed ID: 25142864]. [PubMed Central ID: PMC5012903]. <https://doi.org/10.1002/jcb.24946>.
 20. Liu J, Qian J, Mo Q, Tang L, Xu Q. LncRNA NR2F2-AS1 Silencing Induces Cell Cycle Arrest in G0/G1 Phase via Downregulating Cyclin D1 in Colorectal Cancer. *Cancer Manag Res*. 2020;**12**:1835-43. [PubMed ID: 32210626]. [PubMed Central ID: PMC7073446]. <https://doi.org/10.2147/CMAR.S221996>.
 21. Reis FS, Lima RT, Morales P, Ferreira IC, Vasconcelos MH. Methanolic Extract of *Ganoderma lucidum* Induces Autophagy of AGS Human Gastric Tumor Cells. *Molecules*. 2015;**20**(10):17872-82. [PubMed ID: 26426001]. [PubMed Central ID: PMC6332321]. <https://doi.org/10.3390/molecules201017872>.
 22. Tandel P, Ebrahimi E, Ramzi M, Rezvani A, Ranjbaran R, Tamaddon G. Evaluation of BECN1 Gene Expression in Remission-Induction Therapy of Acute Myeloid Leukemia. *Shiraz E-Med J*. 2020;**21**(8). <https://doi.org/10.5812/semj.96683>.
 23. Cheng Y, Xie P. Ganoderic acid A holds promising cytotoxicity on human glioblastoma mediated by incurring apoptosis and autophagy and inactivating PI3K/AKT signaling pathway. *J Biochem Mol Toxicol*. 2019;**33**(11). e22392. [PubMed ID: 31503386]. <https://doi.org/10.1002/jbt.22392>.
 24. Noorolyai S, Shajari N, Baghbani E, Sadreddini S, Baradaran B. The relation between PI3K/AKT signalling pathway and cancer. *Gene*. 2019;**698**:120-8. [PubMed ID: 30849534]. <https://doi.org/10.1016/j.gene.2019.02.076>.
 25. Giansanti V, Torriglia A, Scovassi AI. Conversation between apoptosis and autophagy: "Is it your turn or mine?". *Apoptosis*. 2011;**16**(4):321-33. [PubMed ID: 21404107]. <https://doi.org/10.1007/s10495-011-0589-x>.
 26. Kang R, Zeh HJ, Lotze MT, Tang D. The Beclin 1 network regulates autophagy and apoptosis. *Cell Death Differ*. 2011;**18**(4):571-80. [PubMed ID: 21311563]. [PubMed Central ID: PMC3131912]. <https://doi.org/10.1038/cdd.2010.191>.