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GNA15 induces drug resistance in B cell acute lymphoblastic leukemia by promoting fatty acid oxidation via activation of the AMPK pathway

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Abstract

The prognosis of B cell acute lymphoblastic leukemia (B-ALL) is poor, primarily due to drug resistance and relapse. Ga15, encoded by *GNA15*, belongs to the G protein family, with G protein-coupled receptors playing a crucial role in multiple biological process. *GNA15* has been reported to be involved in various malignancies; however, its potential role in B-ALL remain unknown. In this study, high expression of *GNA15* in B-ALL was observed in multiple databases. We further confirmed an increased transcriptional level of *GNA15* in newly diagnosed B-ALL patients which was closely correlated with relapse. We showed that *GNA15* promoted cell growth, inhibited apoptosis and enhanced drug resistance in leukemia cell lines. Metabolomics analysis revealed a significant enrichment of fatty acid oxidation (FAO) according to the *GNA15* expression. We further confirmed that *GNA15* could enhance FAO process as evidenced by the upregulation of key molecules involved in FAO including carnitine palmitoyl transferase1 (CPT1), CPT2 and CD36. And inhibition of FAO using etomoxir partially reversed the drug resistance caused by high expression of *GNA15*. Mechanism study showed that *GNA15* promoted FAO by up-regulation of AMPK phosphorylation thus leading to survival advantage in leukemia cells. In conclusion, we observed elevated *GNA15* transcript levels in B-ALL, which were associated with relapse. *GNA15* could induce drug resistance though activation of the AMPK/FAO axis in leukemia cell lines. Targeting *GNA15* and FAO may represent potential therapeutic strategy for improving the prognosis of B-ALL.

Keywords B cell lymphoblastic leukemia · *GNA15* · Fatty acids oxidation · Relapse · Adenosine 5'-monophosphate (AMP)-activated protein kinase

Introduction

B cell acute lymphoblastic leukemia (B-ALL) is a neoplastic disorder characterized by aberrant development and malignant proliferation of lymphocytes, resulting in abnormal hematopoietic cell proliferation and pathological differentiation [1, 2]. Relapse is the leading cause of therapy failure in patients with B-ALL. Therefore, increasing attention has been drawn on elucidating the mechanisms underlying drug resistance and survival in ALL cell [3]. *GNA15* encodes the alpha subunit of G15 protein, which belongs to the G protein family [4]. The concerted action of G proteins and G protein-coupled receptors (GPCRs) enables the transduction of signaling in diverse cellular functions, as well as tumor development and metastasis [5]. *GNA15* exhibits aberrant expression pattern in malignancies such as small intestinal neuroendocrine tumors [6] and pancreatic cancer, promoting tumor cell proliferation, adhesion, and survival under

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conditions of nutrient deficiency [7]. HJM de Jonge et al. have reported a high expression of *GNA15* in CD34 + acute myeloid leukemia (AML) cells, which also predicts poor overall survival in normal karyotype AML [8]. Additionally, we observed significantly elevated levels of *GNA15* expression in bone marrow mononuclear cells (BMMNCs) from minimal residual disease positive (MRD) and relapsed patients with B-ALL.

Recently, aberrant energy metabolism, such as glycolysis and fatty acid oxidation (FAO), have been implicated in the resistance and relapse in hematological malignancies. Significant correlation between FAO and the development of resistance in leukemia cells has been reported [9–11]. Inhibiting FAO has the potential to increase the susceptibility of human AML cells to drugs that induce apoptosis [12].

In our study, we conducted separate metabolomic analyses on *GNA15* gene-modified cell lines. The results indicated significant differences in the metabolome of leukemia cell lines after knockdown or overexpression of *GNA15*, with lipid metabolism being predominantly enriched, including the FAO pathway. Additionally, *GNA15* was found to enhance the resistance of leukemia cells towards commonly used chemotherapeutic agents. Therefore, we propose that *GNA15* may contribute to drug resistance in ALL cells through its regulation of FAO.

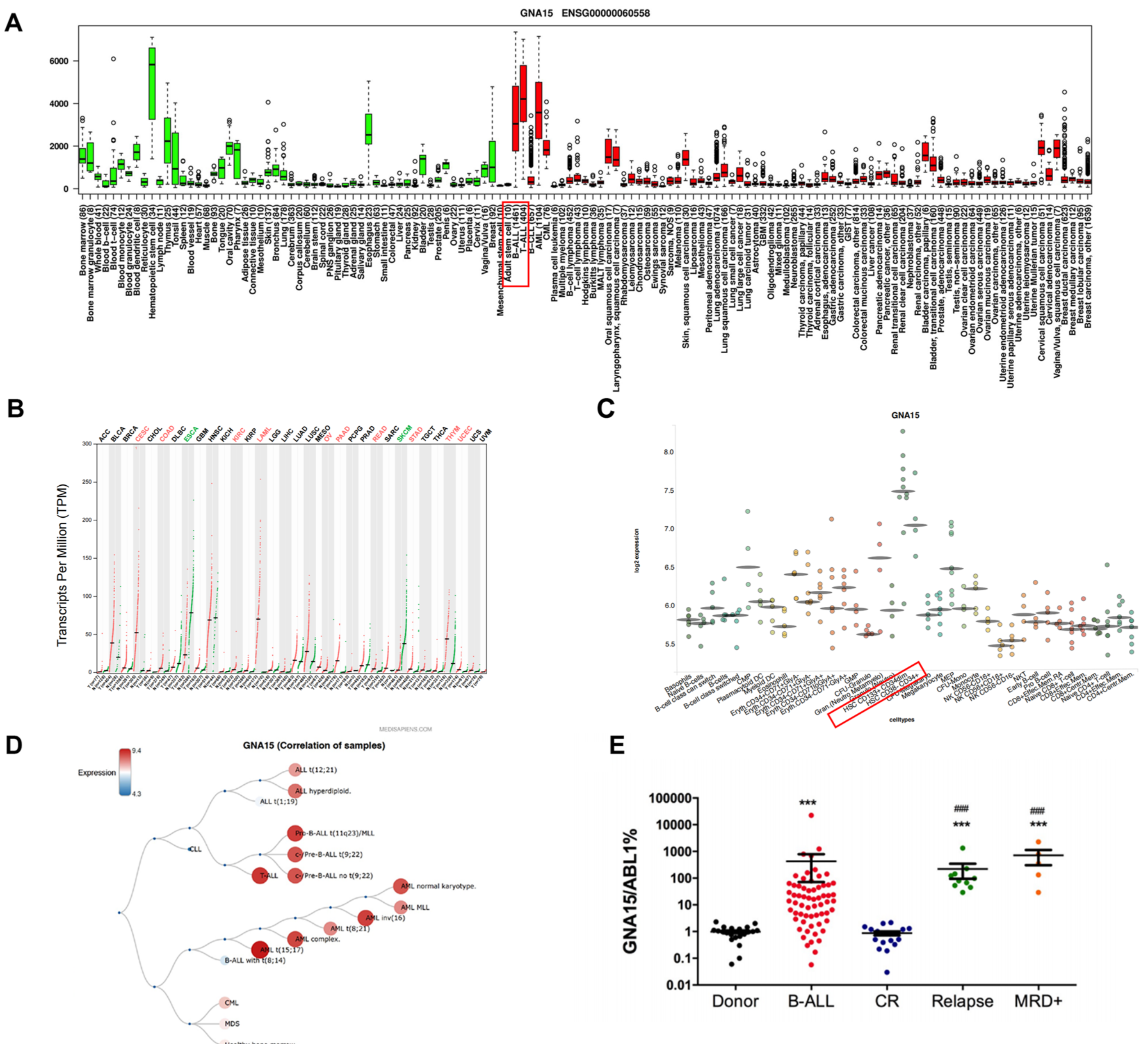


Fig. 1 *GNA15* is selectively highly expressed in acute leukemia cells and hematopoietic stem/progenitor cell. The transcription level of *GNA15* in normal tissues and different tumors in In Silico Transcriptomics database (**A**). Expression profile of *GNA15* across all tumor samples and their corresponding normal tissues (**B**). *GNA15* transcript level in diffrent kinds of blood system associated cells in Blood Spot database (**C**, **D**). The mRNA transcription levels of *GNA15* in healthy donors, newly diagnosed, complete remission, relapsed and MRD + B-ALL patients were detected by RT-qPCR. Transcription level of *GNA15* were normalised to ABL1 expression (**E**). ***, compared with donors, $p < 0.001$; ###, compared with CR, $p < 0.001$

Materials and methods

Leukemia cell lines and reagents

Human leukemia cell lines SUP-B15, BALL-1, Nalm6, KG-1, HL60, K562 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and BV173 was obtained from Guangzhou Jennio Biotech Co. Ltd (Guangzhou, China). RAMOS were purchase from Procell Life Science &Technology Co., Ltd. All these cells were cultured in RPMI-1640 (Corning, Inc.) with 10% FBS (Pansera, ES, Fetal bovine serum, PAN) and 1% penicillin

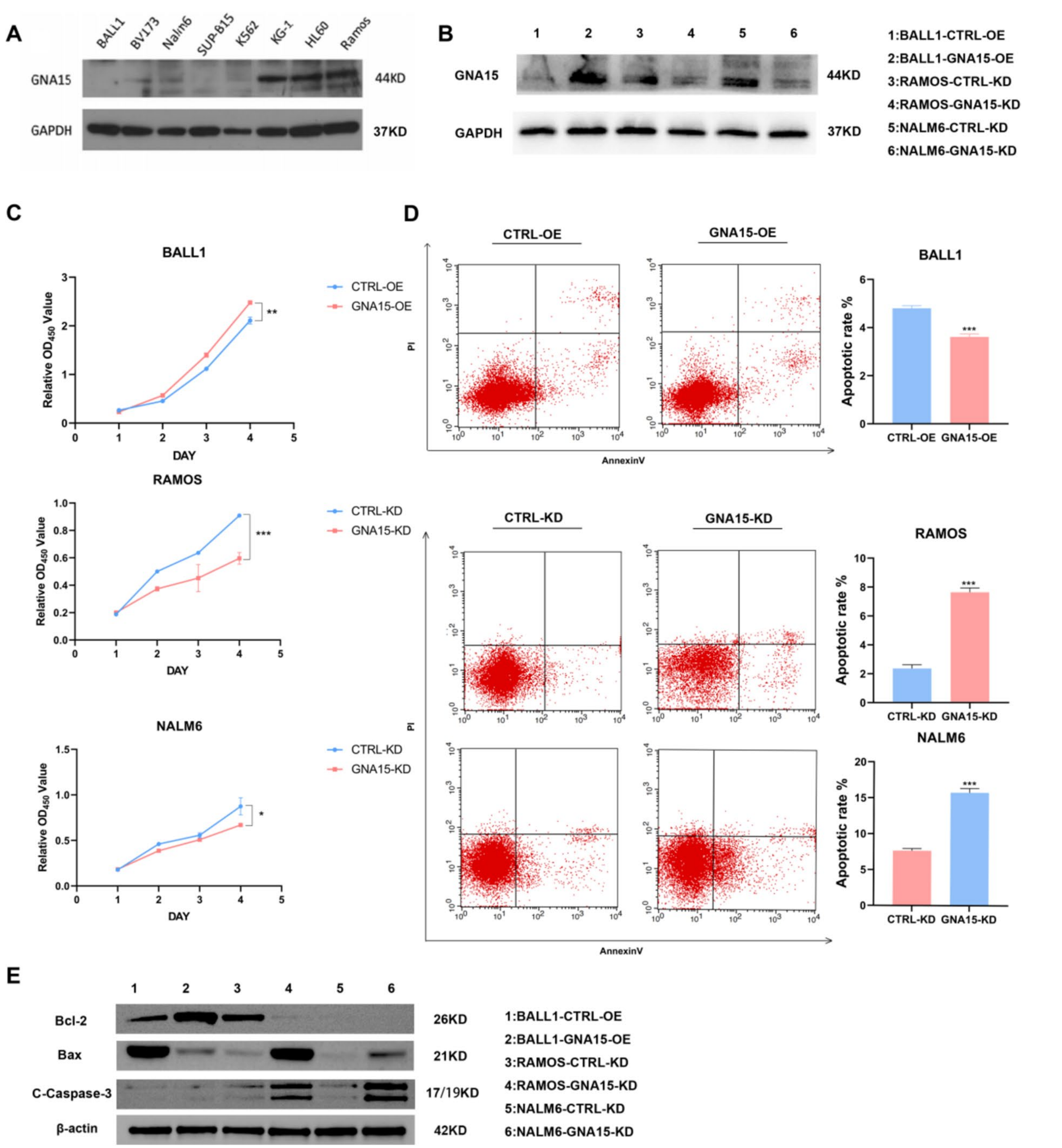


Fig. 2 *GNA15* promotes the proliferative potential and survival of ALL cell lines. The expression level of *GNA15* in leukemia cell lines and efficiency of overexpression and knockdown of *GNA15* in BALL1, RAMOS and NALM6 cell line were verified by western blot analysis (**A**, **B**). Cell proliferation was determined by CCK-8 (**C**). Apoptosis levels detected by Flow cytometry analysis in BALL1 *GNA15*-OE cells,RAMOS *GNA15*-KD cells and NALM6 *GNA15*-KD cells (**D**).*$P<0.05$; **$P<0.01$; ***$P<0.001$.Western blot analysis of the Apoptosis-related protein expression levels of BCL2,BAX and Cleaved-Caspase (**E**)

and streptomycin (C0222, Beyotime Biotechnology), and incubated at 37 degrees Celsius with 5% CO_2.

Lentiviral transduction

BALL-1 cells were infected with lentiviral vector for *GNA15*-overexpression. NALM6 and RAMOS were infected with Lentiviral shRNAs targeting *GNA15* (Shanghai Genechem Co., LTD, MOI = 100). Media-containing lentiviral particles were substituted with complete medium 24 h post-infection. Purinomycin (ST551, Beyotime Biotechnology) with corresponding concentrations for ≥ 6 weeks post-infection was used to construct stably infected cell lines.BALL1 with 0.5 μg/ml, NALM6 and RAMOS with 1.5 μg/ml.

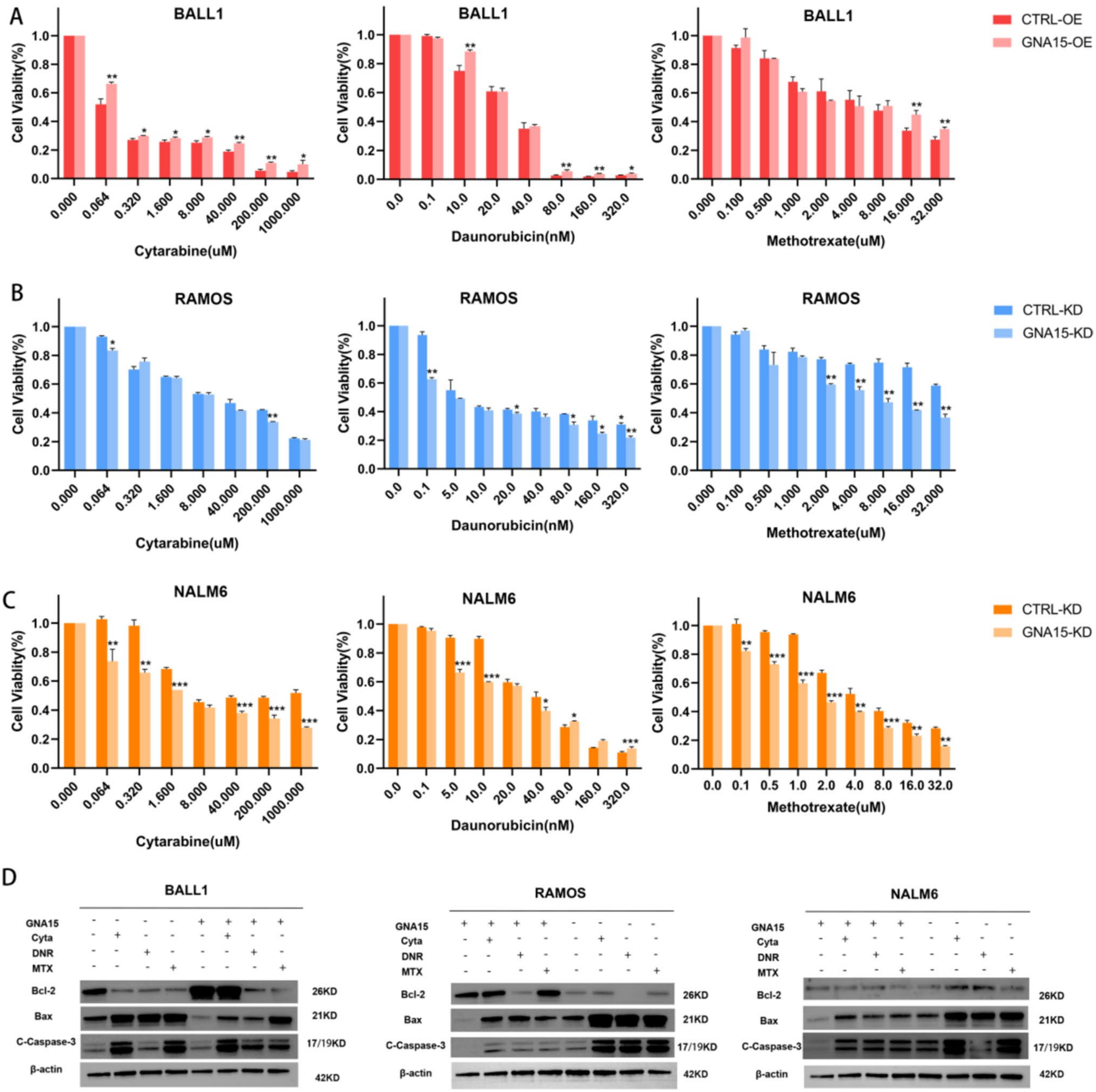


Fig. 3 Overexpression of *GNA15* reduces the sensitivity of leukemia cell lines to antileukaemia drug. *GNA15* overexpression increased proliferation of BALL-1 cells treated with cytarabine, daunorubicin and methotrexate compared with controls (**A**). *GNA15* konckdown decreased the proliferation of RAMOS cells treated with cytarabine, daunorubicin and methotrexate compared with controls (**B**). *GNA15* konckdown reduced the survival of NALM6 cells treated with cytarabine, daunorubicin and methotrexate compared with controls (**C**). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Western blot analysis of the Apoptosis related protein expression levels with treatment of cytarabine, daunorubicin and methotrexate compared with controls (**D**)

RT-qPCR and western blotting were used to verify *GNA15* expression levels.

Clinical samples

Bone marrow samples were collected from newly diagnosed patients with B-ALL ($n=63$) at the Hematology Department of the First Affiliated Hospital of Chongqing Medical University between 2021 and 2023. And the study was approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University (K2023-449).

RNA preparation and RT-qPCR

Mononuclear cells were isolated from bone marrow samples by Ficoll-HypaqueTM density gradient centrifugation. Total RNA was extracted using TRIzol reagent and cDNA was synthesized using RT Master Mix for qPCR II (MCE).PCR was executed using a CFX Connect Real-Time PCR system and SYBR Green qPCR Master Mix (MCE, HY-K0501A) at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The relative mRNA expression were calculated using the $2^{-\Delta\Delta ct}$ method. Primer and probe sequence for *GNA15* were synthesized by Takara Biotechnology Co., Ltd. *GNA15* forward primer:

5'-CAACAATTACATTTCCTGCACCAA-3'; *GNA15* reverse primer: 5'-CCACCTCCTTGATGTGATCCA-3'.

Cell viability

BALL1 cells were seeded in 96-well plates at 5×10^3 cells per well in 100μl culture medium, while NALM6 and RAMOS cells were seeded at 8×10^3 in the same way. Plates were subjected to scanning using a microplate reader at 450 nm at the designated time intervals. The cell viability following drug exposure, including cytarabine (Cyta), daunorubicin (DNR), and methotrexate (MTX), was assessed using the CCK8 assay.

All these drugs were purchased from Med Chem Express company.

Table 1 IC50 of drugs

Drugs / Groups		Cyta(nM)	DNR(nM)	MTX(μM)
BALL-1	CTRL-OE	37.46	23.93	5.432
	GNA15-OE	69.96	26.91	7.063
NALM6	CTRL-KD	131.9	36.87	5.7
	GNA15-KD	16.5	23.4	1.221
RAMOS	CTRL-KD	21.61	12.52	158
	GNA15-KD	13.72	2.167	7.238

Flow cytometry

Apoptosis rate in each group was determined using the Flow cytometry (CytoFLEX; Beckman Coulter, Inc.) to assess Annexin V and 7-ADD double-staining. Approximately 5×10^5 cells were analyzed in each sample following the manufacturer's protocol.

Protein isolation and western blot analysis

Cells were collected, washed three times with PBS and lysed in radioimmunoprecipitation containing phenylmethylsulfonyl fluoride. The protein concentration was measured using a bicinchoninic acid kit (Beyotime Institute of Biotechnology). Total protein (30 μg) was separated on 10 or 12% SDS-PAGE gels and transferred onto PVDF membranes. The western blot was performed as previously described. And the specific antibodies are as follow: *GAPDH, GNA15, CPT1A, CPT2, CD36, ACS, BCL-2, BAX, CASPASE-3, AMPK, p-AMPK, STAT3, p-STAT3*.

Tumour xenograft mouse model

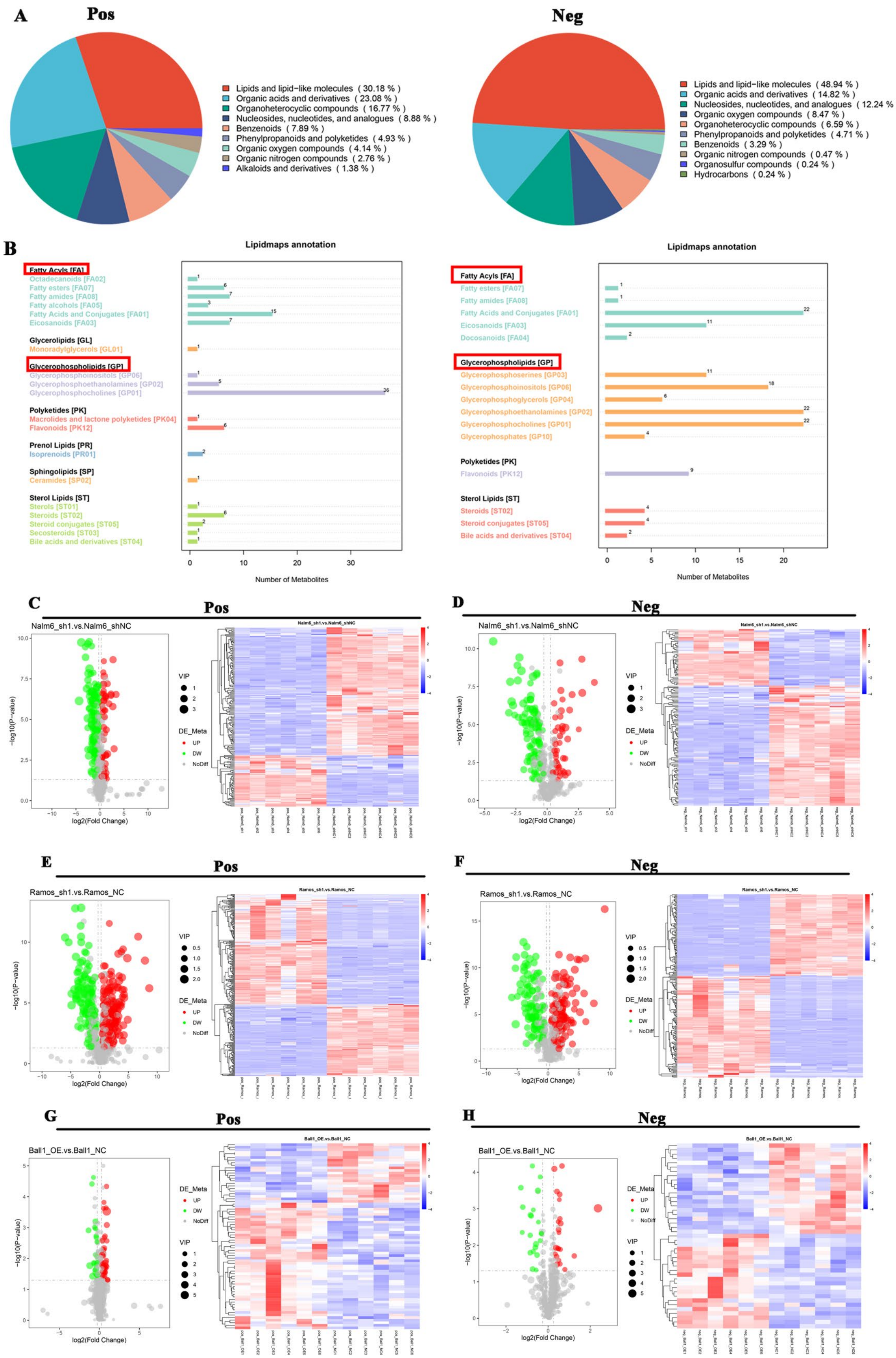
Male athymic Balb/c nude mice, aged 4–5 weeks, and weighing 15–20 g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.BALL1 cells (5×10^6 cells in 0.1 ml of PBS) transduced with the indicated lentivirus were injected subcutaneously into the dorsal right flank area (five mice/group). Tumor diameters and mice weight were measured every 2 days for 15 days. Tumor volume was estimated by measuring the longest and shortest diameters of the tumor as described. Mice were euthanized on day 15 and tumors removed for subsequent experiments. And the tumor was calculated: Volume = (length × width^2)/2. Animal experiments were approved by the Institutional Animal Care and Use Committee of Chongqing Medical University according to the Declaration of Helsinki.

Untargeted metabolomics

Untargeted metabolomics is based on liquid-mass combination (LC–MS) technology [13, 14] for omics research. Nalm6-*GNA15*-KD, Nalm6-CTRL-KD, Ramos-*GNA15*-KD, Ramos-CTRL-KD and BALL1-*GNA15*-OE, BALL1-CTRL-OE cells were collected, and the number of cells was greater than 1×10^7, with 6 repeat samples in each group. And the experiment was carried out by Novogene Technology (Beijing, China).

Statistical analysis

All data were analyzed by GraphPadPrism9.0 and shown as the mean ± SEM. Mann– Whitney U test and Student' t-test


A
Pos
Lipids and lipid−like molecules (30.18 %)
Organic acids and derivatives (23.08 %)
Organoheterocyclic compounds (16.77 %)
Nucleosides, nucleotides, and analogues (8.88 %)
Benzenoids (7.89 %)
Phenylpropanoids and polyketides (4.93 %)
Organic oxygen compounds (4.14 %)
Organic nitrogen compounds (2.76 %)
Alkaloids and derivatives (1.38 %)
Neg
Lipids and lipid−like molecules (48.94 %)
Organic acids and derivatives (14.82 %)
Nucleosides, nucleotides, and analogues (12.24 %)
Organic oxygen compounds (8.47 %)
Organoheterocyclic compounds (6.59 %)
Phenylpropanoids and polyketides (4.71 %)
Benzenoids (3.29 %)
Organic nitrogen compounds (0.47 %)
Organosulfur compounds (0.24 %)
Hydrocarbons (0.24 %)
B
Lipidmaps annotation
Fatty Acyls [FA]
Octadecanoids [FA02]
Fatty esters [FA07]
Fatty amides [FA08]
Fatty alcohols [FA05]
Fatty Acids and Conjugates [FA01]
Eicosanoids [FA03]
Glycerolipids [GL]
Monoradylglycerols [GL01]
Glycerophospholipids [GP]
Glycerophosphoinositols [GP06]
Glycerophosphoethanolamines [GP02]
Glycerophosphocholines [GP01]
Polyketides [PK]
Macrolides and lactone polyketides [PK04]
Flavonoids [PK12]
Prenol Lipids [PR]
Isoprenoids [PR01]
Sphingolipids [SP]
Ceramides [SP02]
Sterol Lipids [ST]
Sterols [ST01]
Steroids [ST02]
Steroid conjugates [ST05]
Secosteroids [ST03]
Bile acids and derivatives [ST04]
Number of Metabolites
Lipidmaps annotation
Fatty Acyls [FA]
Fatty esters [FA07]
Fatty amides [FA08]
Fatty Acids and Conjugates [FA01]
Eicosanoids [FA03]
Docosanoids [FA04]
Glycerophospholipids [GP]
Glycerophosphoserines [GP03]
Glycerophosphoinositols [GP06]
Glycerophosphoglycerols [GP04]
Glycerophosphoethanolamines [GP02]
Glycerophosphocholines [GP01]
Glycerophosphates [GP10]
Polyketides [PK]
Flavonoids [PK12]
Sterol Lipids [ST]
Steroids [ST02]
Steroid conjugates [ST05]
Bile acids and derivatives [ST04]
Number of Metabolites
C
Pos
Nalm6_sh1.vs.Nalm6_shNC
−log10(P−value)
log2(Fold Change)
VIP
DE_Meta
UP
DW
NoDiff
D
Neg
Nalm6_sh1.vs.Nalm6_shNC
−log10(P−value)
log2(Fold Change)
VIP
DE_Meta
UP
DW
NoDiff
E
Pos
Ramos_sh1.vs.Ramos_NC
−log10(P−value)
log2(Fold Change)
VIP
DE_Meta
UP
DW
NoDiff
F
Neg
Ramos_sh1.vs.Ramos_NC
−log10(P−value)
log2(Fold Change)
VIP
DE_Meta
UP
DW
G
Pos
Ball1_OE.vs.Ball1_NC
−log10(P−value)
log2(Fold Change)
DE_Meta
UP
DW
NoDiff
VIP
H
Neg
Ball1_OE.vs.Ball1_NC
−log10(P−value)
log2(Fold Change)
DE_Meta
UP
DW
VIP

◂**Fig. 4** The metabolomic analysis revealed significant variations in the metabolomics of distinct *GNA15*-modified acute leukemia cell lines. The chemical classification of metabolites identified in the project was analyzed (**A**). The KEGG database was utilized for the annotation of the identified metabolites, aiming to comprehend their functional characteristics and classify them accordingly (**B**). Volcanic maps and cluster analysis of total differential metabolites showed the overall distribution of differential metabolites in *GNA15*-OE and *GNA15*-KD cells compared with controls (**C**–**H**). Each point in the volcano map represents a metabolite, significantly up-regulated metabolites are represented by red dots, significantly down-regulated metabolites are represented by green dots, and the size of the dots represents the VIP value.Hierarchical cluster analysis was performed for all the different metabolites between the obtained comparison pairs.Vertical is the cluster of samples, horizontal is the cluster of metabolites

was used for comparison between two groups, and $P < 0.05$ was considered statistically significant. All experiments were repeated three times.

Results

GNA15 exhibits high expression levels in B-ALL and is associated with relapse

To assess the expression of *GNA15* in ALL, we initially examined its expression using three online databases: In Silico Transcriptomics, GEPIA (http://gepia.cancer-pku.cn/) and Blood Spot database (www.bloodspot.eu). Analysis from the In Silico Transcriptomics database and Blood Spot revealed that *GNA15* was prominently expressed in hematopoietic stem cells and acute leukemia (Fig. 1A–C). Additionally, GEPIA data demonstrated elevated expression of *GNA15* in several tumors, particularly LAML (Fig. 1D). Subsequently, we determined the transcription expression level of *GNA15* in BMMNCs derived from ALL patients (Fig. 1E). The results demonstrated an upregulation of *GNA15* expression in B-ALL patients compared to healthy controls, which subsequently decreased to levels consistent with those observed in donors during complete remission (CR). Conversely, elevated levels of *GNA15* were observed during disease relapse or detection of MRD + status among patients, in comparison to the control group. Subsequently, we conducted an analysis on the correlation between *GNA15* and patient prognosis by utilizing the GEO database data set specifically designed for ALL (Figure S1A). In GSE28460, the transcript level of *GNA15* in patients with relapse and early relapse was not statistically significant. Simultaneously *GNA15* transcript level of patients with early recurrence was found to be similar with late relapse. The findings from this section suggest that *GNA15* has the potential to play an important role in occurrence and development of ALL.

GNA15 promotes cellular proliferation and inhibits apoptosis in ALL cell lines

To investigate the role of *GNA15* in ALL cell lines, we initially assessed the protein expression of *GNA15* in acute leukemia cell lines (Fig. 2A). Subsequently, lentivirus vectors were employed to conduct *GNA15*-KD and *GNA15*-OE ALL cell lines. And the efficiency of knockdown and overexpression was confirmed through western blot analysis (Fig. 2B). After that, we conducted an investigation into the potential impact of *GNA15* on the proliferation and apoptosis of ALL cell lines. And the results indicated that *GNA15* overexpression could increase cell proliferation and reduce apoptosis rates in BALL cells. It is, of course, worth noting that given the inherently relatively low apoptosis rate of BALL1 cells in the normal state, the apoptosis rate did not decrease significantly after overexpression of GNA15, although the data reached statistical significance. Knockdown of *GNA15* in NALM6 and RAMOS cells resulted in a significant decrease in proliferation accompanied by increased apoptosis rates (Fig. 2C, D). It was worth noting that the changes in Bax, Bcl2, and C-caspase 3 protein levels were consistent with those in apoptosis rates in each group. Based on the results above, we believe that *GNA15* may a role in promoting cell proliferation and inhibiting apoptosis in ALL cells.

GNA15 enhances drug resistance in ALL cell lines

In order to detect the impact of *GNA15* on anti-leukemia drugs, we conducted CCK-8 assay. ALL cells were treated with cytarabine, daunorubicin, methotrexate in different concentrations, and then measure the OD values. The results showed that the concentration of the drug increased, the cell viability decreased. Besides, the cell viability of the *GNA15*-OE group was higher than that of the control group in BALL1 cell line (Fig. 3A), in contrast, the *GNA15*-KD groups had lower cell viability compared with the control groups in NALM6 and RAMOS cells(Fig. 3B, C). Furthermore, we determined the IC50 values for each group as depicted in Table 1. The IC50 value was higher for the *GNA15*-overexpressing group compared to the CTRL-OE group in BALL1 cells. In contrast, IC50 values were lower for both *GNA15*-knockdown groups compared to their respective control groups in NALM6 and RAMOS cells.

Moreover, we observed alterations in the expression of apoptosis-related proteins across all experimental groups. ALL cells were treated with anti-leukemia drugs in IC50 concentration, respectively. And there was a decrease in the levels of anti-apoptotic protein Bcl2, accompanied by an increase of Cleaved caspase 3 and Bax. In BALL1 cells, *GNA15* overexpression resulted in elevated Bcl2 levels and reduced levels of Cleaved caspase 3 and Bax compared to control group. Moreover, knockdown of *GNA15* resulted

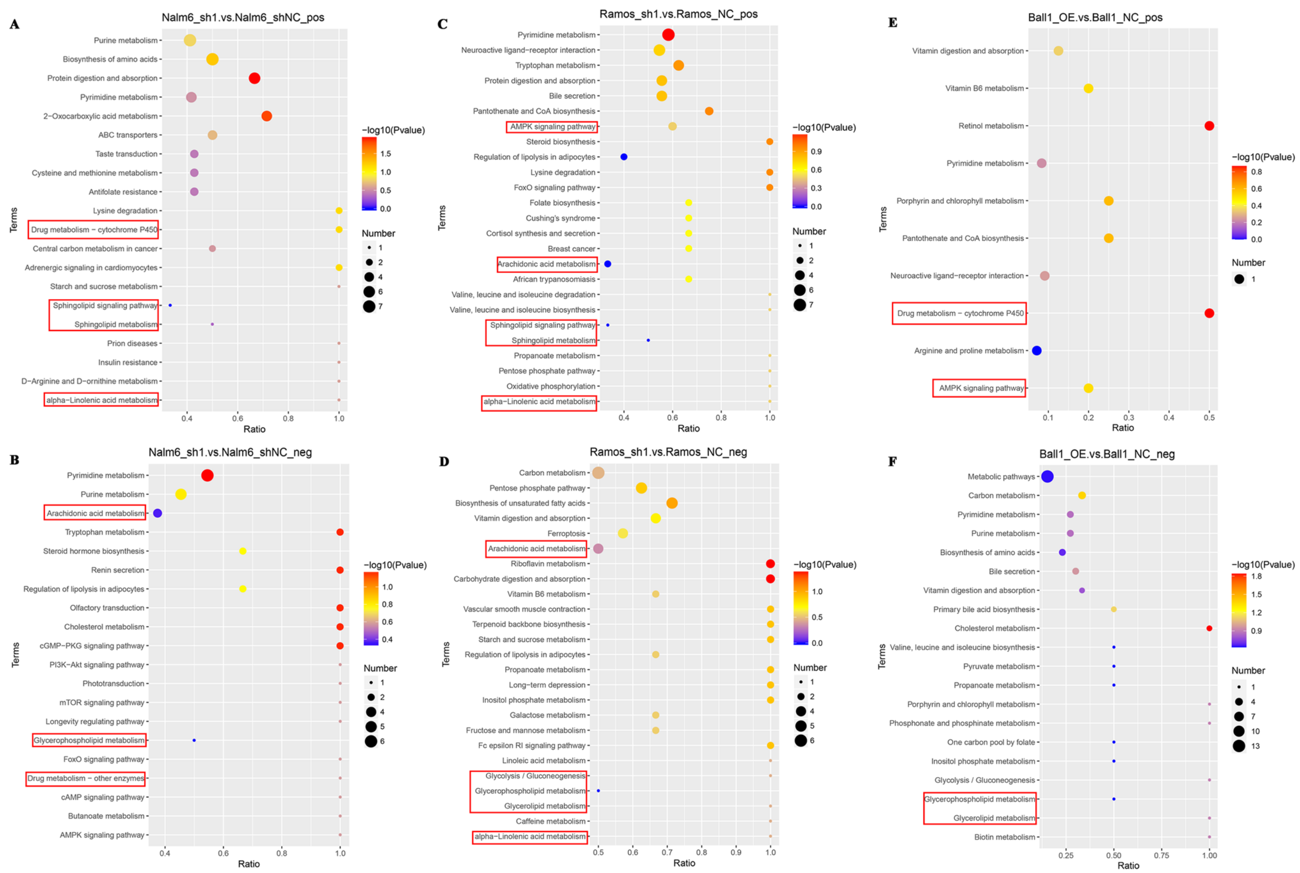


Fig. 5 The different metabolites of ALL cells could be enriched in the FAO-related pathway. KEGG enriched bubble diagram of ALL cell lines, FAO-relative pathway was marked in the diagram (**A**–**F**). In the figure, the horizontal coordinate is x/y (the number of differentiated metabolites in the corresponding metabolic pathway/the total number of identified metabolites in the pathway), and the higher the value, the higher the concentration of differentiated metabolites in the pathway. The color of the dots represents the *P*-value of the hypergeometric test, and the smaller the value, the more reliable and statistically significant the test. The size of the dot represents the number of differentiated metabolites in the corresponding pathway, and the larger the point, the more differentiated metabolites in the pathway

in elevated levels of Cleaved caspase 3 and Bax expression in NALM6 and Ramos cells. However, the impact of *GNA15* on DNR appeared to be minimal (Fig. 3D). To sum up, these findings suggested that overexpression of *GNA15* may reduce the sensitivity of leukemia cells towards anti-leukemia drugs.

GNA15 is involved in the regulation of the fatty acid oxidation process

In order to further investigate how *GNA15* affects the process of anti-leukemia drugs resistance in ALL cell lines. The intracellular metabolites and supernatants of 4 cell lines were detected by LC–MS metabolomics (HL60-CTRL, HL-60-*GNA15*-KD, BALL1-CTRL, BALL1-*GNA15*-OE). The results of supervised multidimensional statistical methods, namely partial least square discriminant analysis and correlation analysis, indicated substantial variations in metabolites across the groups (Fig. S2). In the purpose of getting more concrete results, we conducted Untargeted metabolomics on NALM6, RAMOS and BALL1 cell lines ($n=6$, respectively). A total of 813 positive mode metabolites and 532 negative mode metabolites were detected, of which 30.18% positive and 48.94% negative were lipids and lipid-related molecules (Fig. 4A). At the same time, the results of lipid maps analysis showed that both positive and negative metabolites could be classified into fatty acyl and glycerophospholipid (Fig. 4B). In addition, the detected metabolites were annotated by KEGG, and the results showed that metabolites may be related to cell growth and death, tumor drug resistance, and lipid metabolism (Fig. S3). After that, differential metabolites were identified based on the following criteria: VIP > 1.0, FC > 1.2 or FC < 0.833 and *P*-value < 0.05. A total of 54 metabolites exhibited up-regulation, while 134 metabolites showed down-regulation in the Nalm6 cell group. Additionally, there were 44 up-regulated and 94 down-regulated negative metabolites detected. (Fig. 4C, D). Similarly, we identified 183 up-regulated and 118 down-regulated positive metabolites, along with

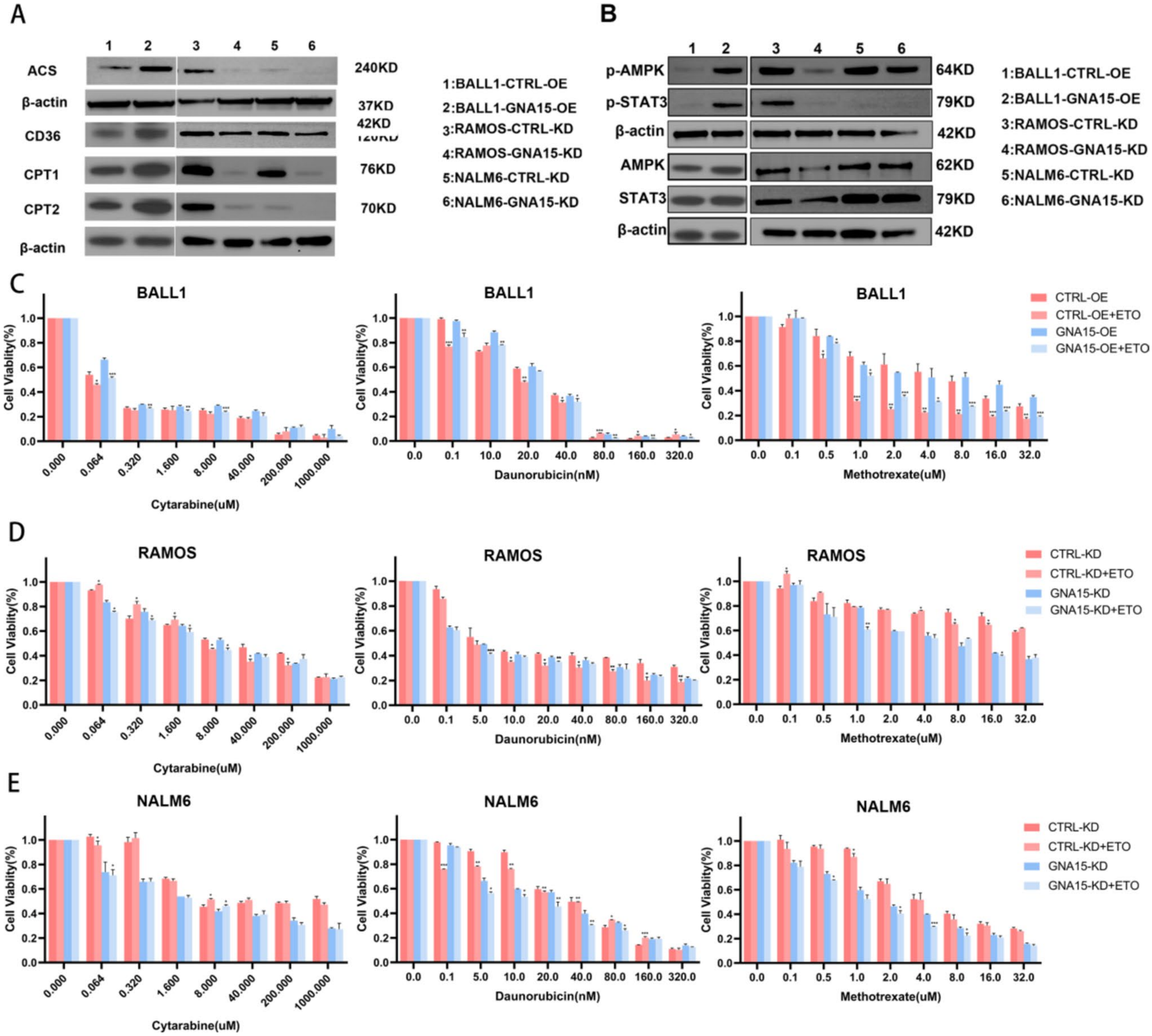


Fig. 6 *GNA15* enhances anti-leukemia drugs resistance through up-regulation of FAO. Western blot analysis of FAO-correlated and AMPK/STAT3-related proteins in *GNA15*-OE and *GNA15*-KD cells and controls (**A**, **B**). FAO inhibitor etomoxir reverses the decreased sensitivity of leukemia cell lines to antileukemia drug caused by overexpression of *GNA15*.The drug sensitivity of BALL1 cells was diminished upon overexpression of *GNA15*, resulting in reduced responsiveness to cytarabine, daunorubicin, and methotrexate, the utilization of FAO inhibitor Etomoxir further attenuated the drug sensitivity of BALL1 cells (**C**).The drug sensitivity test indicated that RAMOS and NALM6 were more sensitive to cytarabine, daunorubicin, and methotrexate after *GNA15* knockdown, and the drug sensitivity of RAMOS and NALM6 cells could be increased by using FAO inhibitor Etomoxir (**D**, **E**). Flow cytometry analysis showing apoptosis rate in BALL1, RAMOS, NALM6 cells with treatment as indicated (**F–H**).$*P<0.05$; $**P<0.01$; $***P<0.001$

113 up-regulated and 91 down-regulated negative metabolites in the Ramos group (Fig. 4E, F). Lastly, in BALL-1 cells, a total of 49 up-regulated, 23 down-regulated positive metabolites and 22 up-regulated, 21 down-regulated negative metabolites were detected (Fig. 4G, H). KEGG enrichment analysis was performed for these differential metabolites. Although the enrichment pathways were different in the three cell lines, some FAO-related pathways could be enriched in all of them. The positive metabolites of Nalm6 group were enriched to alpha-Linolenic acid metabolism, Sphingolipid metabolism, and Sphingolipid signaling pathway, meanwhile negative metabolites were enriched to Arachidonic acid metabolism and Glycerophospholipid metabolism (Fig. 5A, B). In Ramos cell group, the positive metabolites were enriched to Sphingolipid signaling pathway, Arachidonic acid metabolism and Sphingolipid metabolism, as well as negative metabolites enriched

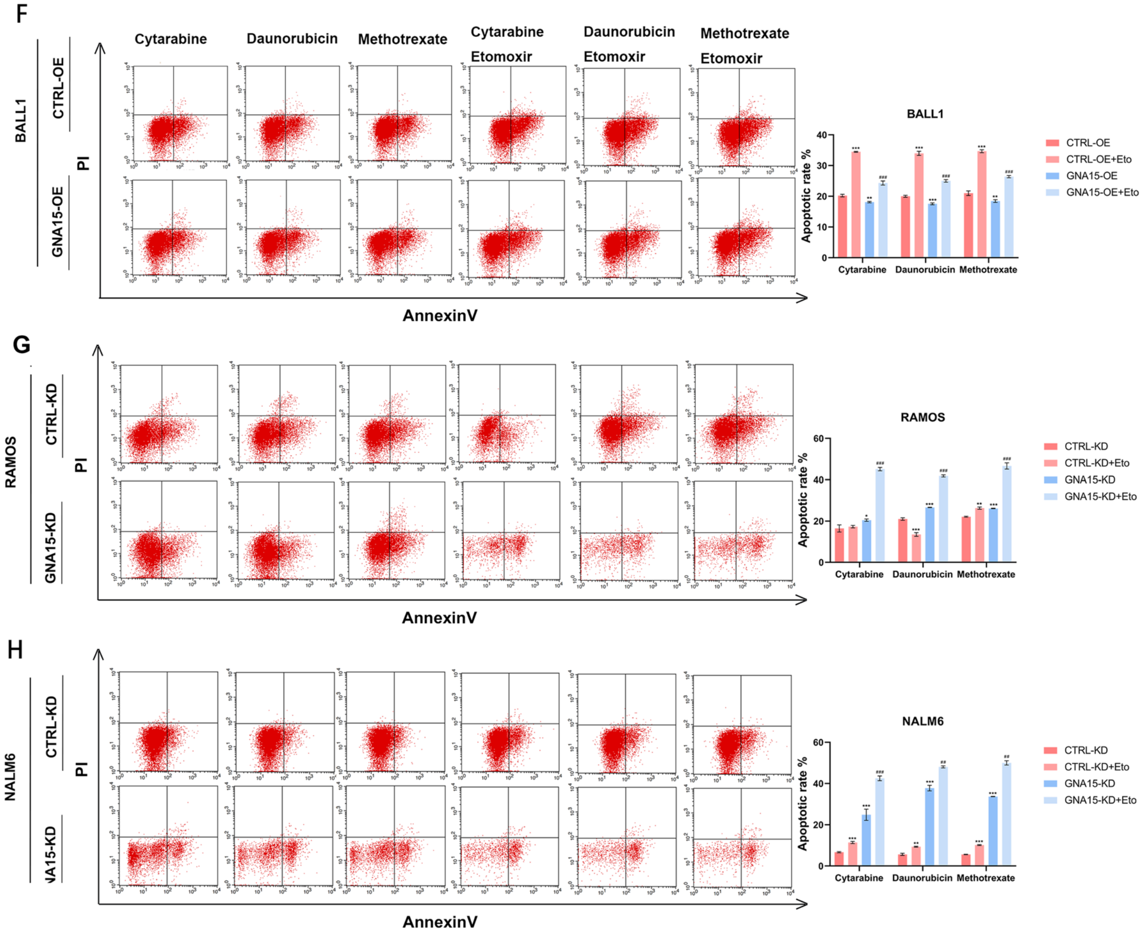


Fig. 6 (continued)

to Glycerophospholipid metabolism and Glycerolipid metabolism (Fig. 5C, D). Finally, in Ball1 cells, there were no FAO-related enrichment pathway for positive metabolites, but negative metabolites were enriched to Glycerolipid metabolism and Glycerophospholipid metabolism (Fig. 5E, F). In summary, the three ALL cells were co-enriched to Glycerophospholipid metabolism. In addition, in Ramos and Nalm6 cells, differential metabolites may be also related to Sphingolipid signaling pathway, Arachidonic acid metabolism and Sphingolipid metabolism.

GNA15 enhances anti-leukemia drugs resistance through up-regulation of FAO

The high expression of *GNA15* significantly enhanced the key molecule of FAO process, such as carnitine palmitoyl transferase1 (CPT1), CPT2, and fatty acid transporter CD36 (Fig. 6A) and up-regulated the phosphorylation levels of AMPK in leukemia cell lines (Fig. 6B). After the treatment of drugs and FAO inhibitore tomoxir (Eto), CCK8 was used to detect the cell viability. The findings suggest that the overexpression of *GNA15* may counteract the reduction in cell viability induced by anti-leukemia drugs in BALL1 cells (Fig. 6C), while *GNA15* knockdown played a cooperative role in reducing cell viability in NALM6 and RAMOS cells (Fig. 6D, E). When it came to FAO, Eto could further reduce cell viability. Consistent results also be found at the IC50 of each group (Table 2). And then, we measured cell apoptosis rates in different groups. On one hand, *GNA15*-OE could decrease the apoptosis rate induced by anti-leukemia drugs in BALL1 cells (Fig. 6F). On the other hand, *GNA15*-KD increase the apoptosis rate induced by anti-leukemia drugs, except for DNR, in NALM6 and RAMOS cells (Fig. 6G, H). Besides, Eto combined with drugs showed the higher apoptosis rate compared with drugs treatment along. The results indicated that *GNA15* may reduce the anti-leukemia drugs susceptibility through FAO process in ALL cells.

GNA15 regulates FAO process by activating AMPK signaling pathway

The KEGG enrichment analysis of the untargeted metabolomics also suggested differential metabolites of ALL cell lines was enriched in AMPK signaling pathway, which is a key signaling pathway for regulating FAO process (Fig. 5C). Later we measured cell apoptosis rates after we treated *GNA15*-OE cells with AMPK inhibitor Dorsomorphin-2HCl and *GNA15*-KD cells with AMPK agonist GSK621. We found inhibition of AMPK pathway could reverse the decrease of apoptosis rate induced by overexpression of *GNA15* (Fig. 7A). Similarly, activation of AMPK pathway could reverse the increase of apoptosis rate induced by knockdown of *GNA15* (Fig. 7B, C). The western blot showed inhibition of AMPK pathway could suppress the key molecule of FAO process, CPT1, CPT2 and CD36 (Fig. 7D). Inversely activation of AMPK pathway could enhance the key molecule of FAO process (Fig. 7E, F). The results suggested that *GNA15* may regulate the fatty acid oxidation process by activating AMPK signaling pathway.

GNA15 promotes ALL growth and increases methotrexate tolerance in vivo

Xenograft mouse model was performed with CTRL-OE and *GNA15*-OE of BALL1 cells to validate the role of *GNA15* on growth and methotrexate effect in vivo. Tumor tissues ($n=5$, respectively) were taken 15 days after cell injection. *GNA15* overexpression significantly increase the tumor volume compared with CTRL-OE group (Fig. 8A). And IHC results presented that the positive intensity of *GNA15*, CPT1, and CPT2 in the *GNA15*-OE group was higher than that in the control group. This indicated that *GNA15* also related to FAO in vivo (Fig. 8B). To verifying the impact of *GNA15* on anti-leukemia drug in vivo, mice were intraperitoneally injected with MTX and Eto, and observed tumor size. As shown in Fig. 8C, the tumor volume of mice treated with MTX was significantly reduced compared to that of untreated mice (Fig. 8C). Furthermore, the combined treatment of *GNA15*-OE and MTX resulted in a further decrease in tumor volume compared to the control group combined with MTX. Besides, Eto was able to reverse the reduction in tumor volume caused by MTX.

Then, we detect the expression of *GNA15*, CPT1 and CPT2 in each group by IHC. And the results showed that Eto could reduce CPT1 and CPT2 expression (Fig. 8D). Therefore, *GNA15* overexpression may play a role in cell growth and MTX tolerance through FAO process in vivo.

Discussion

ALL is a hematologic malignancy characterized by significant biological and clinical heterogeneity, posing a substantial threat to human health [1]. With the gradual understanding of the pathogenesis and pathological process of the disease, as well as the establishment of stratified therapy, targeted drugs, and immunotherapy, there have been improvements in the prognosis of ALL. Consolidation chemotherapy has shown response rates ranging from 74 to 93% [15, 16]. Despite these advancements, the long-term prognosis for adult ALL remains suboptimal, with a 5-year survival rate ranging from approximately 20 to 40% [17, 18]. Relapse following initial remission remains the primary obstacle to effective treatment and long-term survival in adult ALL patients [19–21], MRD cells surviving chemotherapy being considered as potential sources for relapse [22, 23]. Currently, our understanding of molecular mechanisms and regulatory processes underlying ALL progression and relapse is incomplete. Given these limitations in current treatments, it is crucial to conduct research aimed at identifying new molecular markers and therapeutic targets for ALL while also developing novel treatment drugs that can further enhance patient survival rates. In our study findings indicate high expression levels of *GNA15* in ALL patients particularly those with MRD + or relapse. These results prompted us to investigate whether *GNA15* may be associated with ALL relapse through subsequent experiments. Alterations in cell energy metabolism patterns serve as key indicators for tumor cells playing precise regulatory roles in drug resistance invasion and tumor recurrence [24, 25]. The Warburg effect was initially reported as a characteristic metabolic feature observed in tumors which drives cancer cell proliferation aggressiveness potentially contributing to chemotherapy resistance [26, 27]. Revealed by advancing research, the significance of metabolic routes like FAO in drug resistance and relapse of AML has gradually emerged [28, 29]. Recent studies have implicated *GNA15* in cellular energy metabolism.

Table 2 IC50 of drugs with Eto

Drugs		Cyta(nM)	DNR(nM)	MTX(μM)
Groups				
BALL-1	CTRL-OE + Eto	16.03	21.16	0.8409
	GNA15-OE + Eto	31.8	23.07	1.585
NALM6	CTRL-KD + Eto	0.2612	33.72	1.919
	GNA15-KD + Eto	15.14	12.93	1.183
RAMOS	CTRL-KD + Eto	14.02	4.186	25.25
	GNA15-KD + Eto	7.26	0.9464	7.346

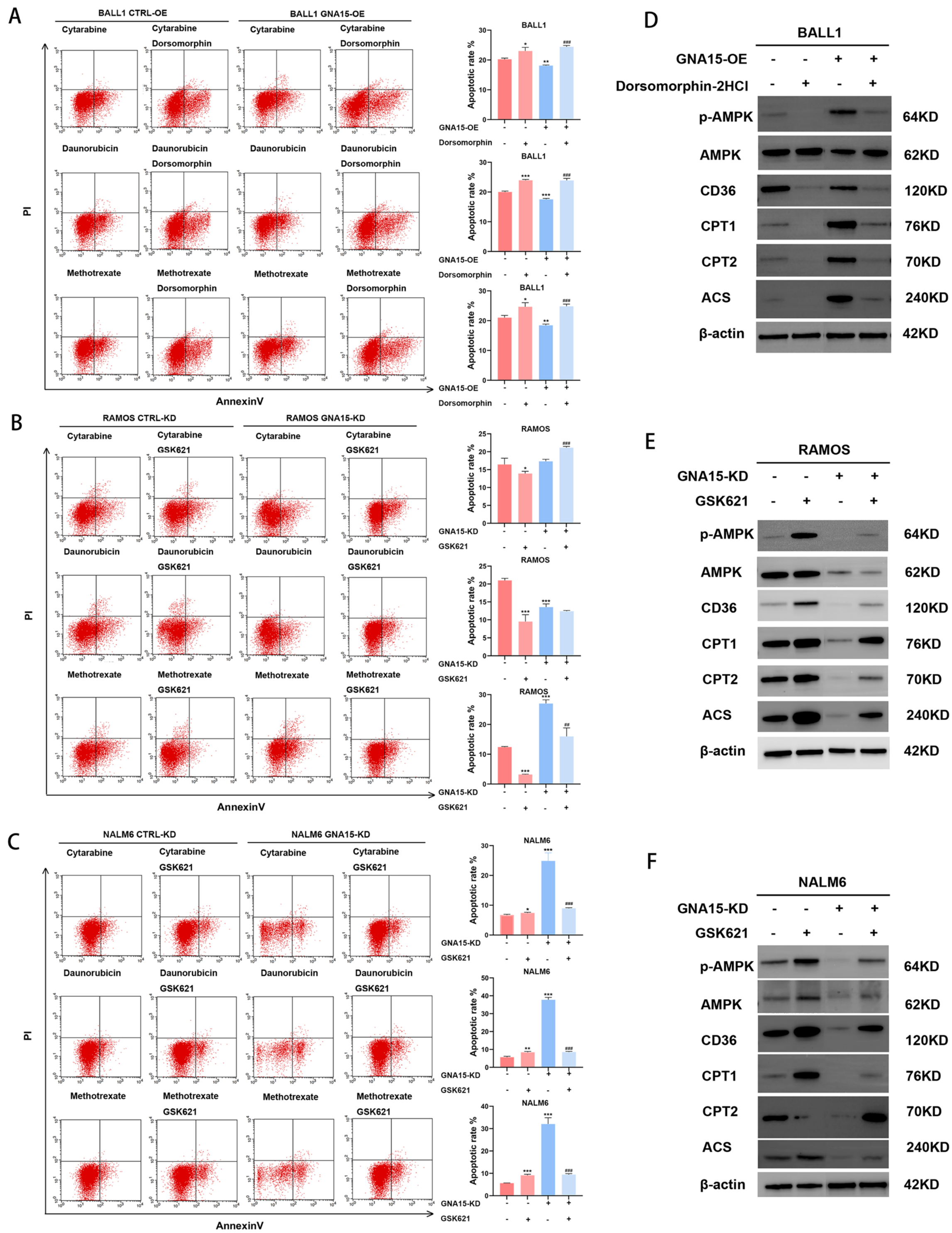


Fig. 7 *GNA15* activates the FAO process in a AMPK phosphorylation-dependent manner. Flow cytometry analysis showing apoptosis level determined subsequent to treatment with cytarabine, daunorubicin, and methotrexate, follwed by AMPK inhibitors Dorsomorphin-2HCl or not in *GNA15*-OE BALL1 cells compared with controls (**A**). Flow cytometry analysis showing apoptosis level determined subsequent to treatment with anti-leukemia drugs, followed by AMPK agonist GSK621 or not in *GNA15*-KD RAMOS and NALM6 cells compared with controls (**B**, **C**). Relative p-AMPK, AMPK, CD36, CPT1, CPT2, ACS were measured by western blot analysis (**D**–**F**). $*P<0.05$; $**P<0.01$; $***P<0.001$

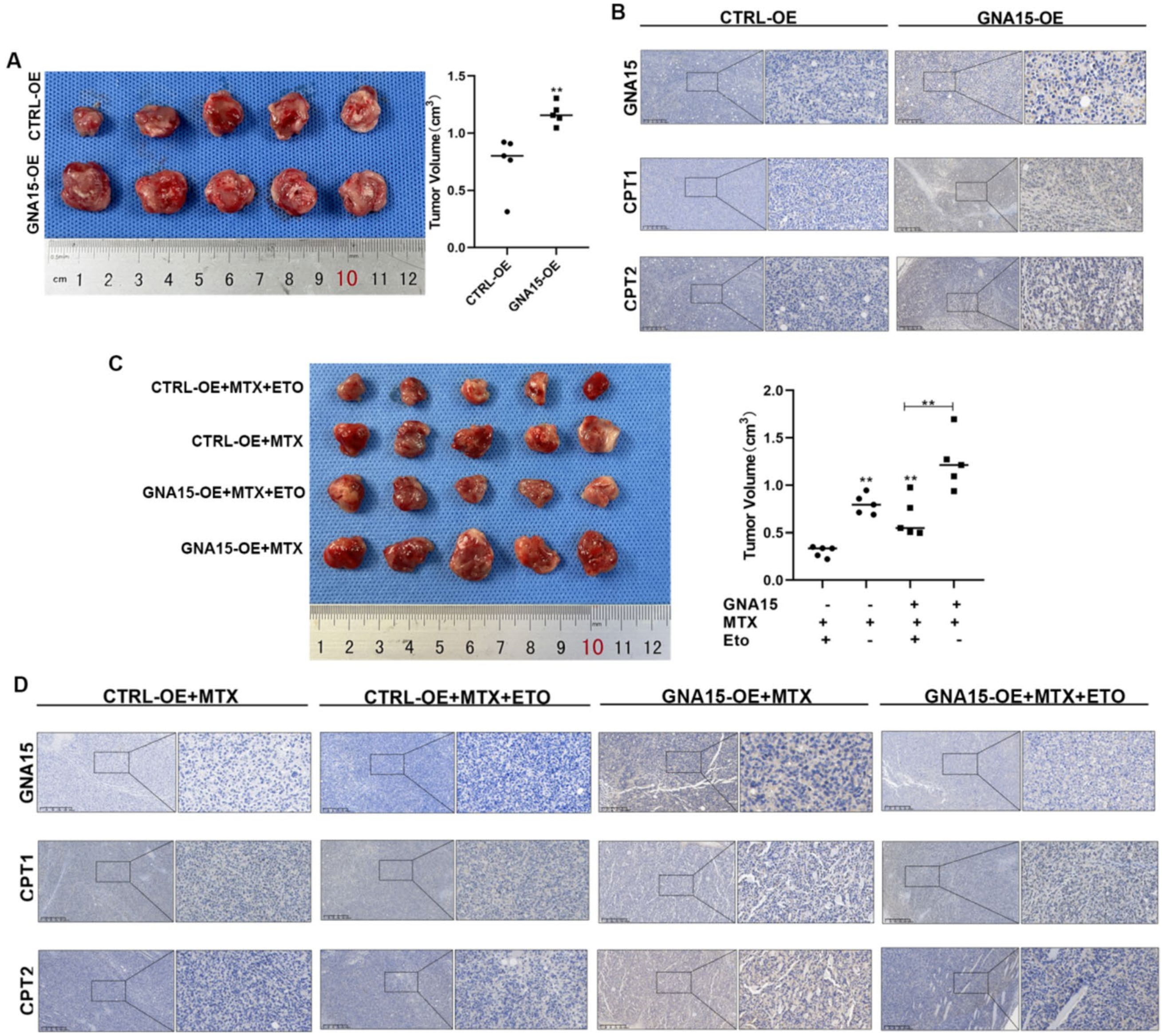


Fig. 8 The overexpression of *GNA15* enhances the in vivo growth and methotrexate tolerance of BALL1 cells. The growth of xenografted tumors of *GNA15*-OE BALL1 cells with treatment as indicated (**A**, **C**).Tumor immunohistochemistry showed the expression of *GNA15*, CPT1 and CPT2 with treatment as indicated (**B**, **D**). $*P<0.05$; $**P<0.01$; $***P<0.001$

For instance, Zeng et al. demonstrated that regulation of *GNA15* by exosomal miR-211-5p influences glucose metabolism, pyroptosis, and the immune microenvironment in melanoma [30]. Furthermore, Li et al. discovered elevated expression levels of *GNA15* in AML compared to the normal control group; Patients showing elevated levels of GAN15 expression demonstrated reduced rates of overall survival and relapse-free survival, whereas *GNA15* facilitated the proliferation of AML cells [31].

In our study, it appears that the modulation of crucial apoptosis-related proteins such as BAX, Cleaved caspase 3, and BCL-2 is influenced by *GNA15*. Overexpression of *GNA15* results in a reduction of BAX levels and an elevation in Cleaved caspase 3 and BCL-2 expression. These alterations imply that *GNA15* might impede the endogenous apoptotic pathway controlled by the BCL-2 protein family, wherein BAX assumes a pro-apoptotic role and BCL-2 serves as an anti-apoptotic [32]. Therefore, upregulation of BCL-2 coupled with downregulation of BAX would potentially impede apoptosis induction. When assessing apoptosis rates under leukemia drug influence, we observed that overexpression of *GNA15* attenuates drug-induced increase in apoptosis whereas silencing *GNA15* exhibits opposite effects. This implies that *GNA15* might interfere with leukemia drugs' ability to induce apoptosis in ALL cells thereby rendering them more resistant to cytotoxic effects exerted by these drugs. Additionally, we have identified associations between *GNA15* and regulation FAO along with metabolic pathways. Given the association of FAO with leukemia cell resistance and resistance to apoptosis-inducing drugs, we postulated that the influence of *GNA15* on FAO and its related pathways might contribute to the observed resistance in ALL cells. As previously mentioned, elevated expression of *GNA15* appears to modulate ALL cell proliferation and resistance by regulating the AMPK/STAT3 pathway. AMPK, an energy-sensing kinase capable of influencing diverse cellular processes such as metabolism and apoptosis, could be affected by *GNA15*-mediated modulation of this pathway, thereby impacting the sensitivity of ALL cells towards apoptosis-inducing signals. Consequently, our final conclusion is that *GNA15* potentially regulates FAO through the AMPK pathway, consequently promoting rapid proliferation in residual ALL cells which may ultimately lead to drug resistance and relapse in acute leukemia.

Ethical approval

The study was approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University, and informed consent was obtained according to the Declaration of Helsinki.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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