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Endothelial lipase promotes acute myeloid leukemia progression through metabolic reprogramming

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Metabolic reprogramming occurs in the clonal evolution of acute myeloid leukemia (AML), which contributes to cell survival under metabolic stress and the development of drug resistance. Leukemic cells exhibit various metabolic profiles, which involve multiple metabolic pathways due to the heterogeneity of AML. However, studies on metabolic targets for AML treatment are mostly focused on glycolysis at present. In this work, we established conditional knock-in AML mouse models harboring Dnmt3aR878H/WT, NrasG12D/WT, and both of the mutations, respectively. Transcriptomic analysis of Gr1+ cells from bone marrow was performed afterward to screen interested metabolic pathways and target genes. Candidate genes were studied using the CRISPR/Cas9 technique, quantitative real-time RT-PCR, and flow cytometric analyses. We revealed that multiple metabolic pathways were affected in AML mice, including lipid metabolism. Endothelial lipase (LIPG) was obviously upregulated in leukemic cells from AML mice with Dnmt3a mutation. We performed knockout of LIPG in OCI-AML3 cells carrying DNMT3A R882C mutation by using the CRISPR/Cas9 technique. Depletion of LIPG led to proliferation inhibition, apoptosis, damage of antioxidant capacity, and myeloid differentiation in OCI-AML3 cells. LIPG might serve as a potential metabolic target for the treatment of AML with abnormal lipid metabolism.

Key words: endothelial lipase, acute myeloid leukemia, lipid metabolism, DNA methyltransferase 3A

Metabolic reprogramming has been widely observed in multiple types of cancers. It confers cancer cells the ability to proliferate and survive, even under nutrient stress or chemotherapy. It has been reported that reprogrammed metabolism can promote proliferation and drug resistance in acute myeloid leukemia (AML). Enhanced aerobic glycolysis is common in various cancer cells including AML blasts [1, 2]. Exogenous glucose analogs, such as 2-deoxy-D-glucose, could sensitize leukemic cells to arabinofuranosylcytidine [3]. Another strategy to affect glycolysis is restraining lactate clearance in leukemic cells. Inhibition of monocarboxylate transporter MCT1 and MCT4 could lead to cell death and increased chemotherapy sensitivity [4]. In face of glucose insufficiency, AML cells could be rewired to consume fructose by upregulating GLUT5-encoding gene SLC2A5 [5]. In addition, various metabolic pathways coexist and cooperate in the process of leukemogenesis [6]. Increased glycolysis doesn't necessarily lead to decreased tricarboxylic acid cycle and oxidative phosphorylation. The acquired metabolic plasticity of leukemic cells enables them to switch to an alternative source of energy, such as shifting from pyruvate oxidation to fatty acid oxidation (FAO) [7]. Lipid biosynthesis and lipolysis are enhanced in various cancer cells [8]. Besides their role in energy supply, lipids are crucial compositions of the cell membrane. Lipid precursors could also contribute to tumor proliferation, anti-apoptosis, and resistance to chemotherapeutics [2, 9]. In the sera of AML patients with a poor prognosis, 11 kinds of fatty acids were decreased because of a larger consumption of lipids [3]. Upregulation of FAO could induce resistance to Venetoclax with azacytidine [10]. By inhibiting fatty acid oxidation with Etomoxir (carnitine palmitoyltransferase 1 inhibitor) or Avocatin B (an odd-numbered carbon lipid derived from avocado fruit), leukemic cells became less resistant to anti-apoptosis drugs or Ara-C [7, 11]. Studies on inhibitors targeting lipid metabolism, especially clinical studies, are still lacking. New targets of different metabolic pathways and more strategies of drug combination are worth studying.

Genetically engineered mouse models enable us to study the mechanism of human leukemogenesis. DNA

methyltransferase 3A (DNMT3A) mutation occurs in about 20–25% of AML patients. DNMT3A mutation has been identified as a driver mutation in the carcinogenesis of AML and often coexists with other genetic abnormalities, such as FLT3, IDH1/2, NPM1, and RAS mutations [12–14]. In our previous work, we established a conditional knock-in (KI) mouse model harboring Dnmt3aR878H/WT, which exhibited moderate AML after induction [15]. Then, we revealed that cooperation of Dnmt3aR878H/WT mutation with NrasG12D/WT mutation could induce severe AML by generating a double knock-in (DKI) mouse model [16]. In this work, we compared the Gr1+ cells of the DKI mice harboring both Dnmt3aR878H/WT and NrasG12D/WT mutations and wild type (WT) mice through RNA-sequencing. We found that AML mice with both Dnmt3aR878H/WT and NrasG12D/WT mice exhibited remodeled metabolism through various metabolic pathways. Endothelial lipase (LIPG) was obviously upregulated in leukemic cells of AML mice harboring DNMT3A mutation. We proved that LIPG might serve as a potential metabolic target for AML treatment.

Materials and methods

Generation of AML mouse models and transcriptomic analysis. The conditional knock-in AML mouse models harboring Dnmt3aR878H/WT, NrasG12D/WT, and both mutations were established as previously described [16]. Mice were used according to animal care standards. All protocols were approved by the Committee on Animal Use for Research at the Shanghai Jiao Tong University School of Medicine. Through intraperitoneal injection of 250 μg polyinosinic-polycytidylic acid (pIpC) every other day for two times in C57 mice at 4 weeks old, the Cre expression was induced. The mice exhibited leukemic phenotypes and were euthanized 4 months after the pIpC injection. Gr1+ cells from the bone marrow of each group were sorted for RNA-sequencing as previously described [16]. Gene Ontology (GO) functional analysis was performed and visualized using R packages including ClusterProfiler version 3.14.3 and GOplot. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment was performed on ClueGO in Cytoscape.

Gene knockout with CRISPR/Cas9 technique. Lentivirus was produced by a co-transfecting lentiGuide-Puro plasmid with packaging plasmids pVSVg and psPAX2 in HEK293(F)T cells. OCI-AML3 cells were transduced to stably express Cas9. Guide RNAs (gRNAs) targeting common exon 2/4 of human LIPG were obtained from www.atum.bio, and were indicated to have a high excision efficiency and low off-target effect. After inserting gRNA into the lentiGuide-Puro vector, subsequently generated lentivirus was transduced into previously manufactured OCI-AML3Cas9 cells to excise LIPG exon regions. Finally, monoclonal cells were amplified and examined for knockout efficiency at DNA and protein level. gRNA1: caccgGAAGGATGCTACCTCTCCGT; aaacACGGAGAGGTAGCATCCTTCc; gRNA2: caccgAACTTCGTGAAAGGAACGGT; aaacACCGTTCCTTTCACGAAGTTcPCR primer: F-CAGGTCTCTCACTCCGCAA; R-ATATACTGCGGGCCTATGCGA.

Western-blot analyses. Cells were collected and total protein was extracted using ProtLytic Protein Lysis and Sample Loading (New Cell & Molecular Biotech). Antibodies used for western blot were anti-β-actin (CST 4970) and anti-LIPG (Absin 115832). Protein extracts were separated on 12% ExpressPlus PAGE Gels (GeneScript) and transferred into polyvinylidene fluoride membranes. After blocking with 5% nonfat milk, PVDF membranes were incubated with primary and then secondary antibodies. At last, visualization of protein bands was performed.

Quantitative real-time RT-PCR (qRT-PCR). Total RNA was extracted from cells using TRIzol Reagent (Invitrogen). Next, Hifair® III 1st Strand cDNA Synthesis SuperMix for qPCR (Yeasen) was used for cDNA synthesis following the manufacturer's instruction. Reagents for qPCR (Hieff TM qPCR SYBR® Green Master Mix) were also purchased from Yeasen. Reactions were performed on Applied Biosystems ViiATM 7 Real-Time PCR System according to the manufacturer's protocol. Log2foldchange was calculated and analyzed by using Prism GraphPad software (La Jolla) and Microsoft Excel (Microsoft).

Cell proliferation, apoptosis, and differentiation assay. OCI-AML3 (DSMZ Cat# ACC 582), U937 (DSMZ Cat# ACC 5), and Kasumi-1 (DSMZ Cat# ACC 220) cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). XEN445 (Cat# HY-12246) was purchased from MedChemExpress and dissolved in dimethylsulfoxide (DMSO) for further experiments. Cell Counting Kit-8 (New Cell & Molecular Biotech) and Annexin V-FITC/PI apoptosis kit (Biolegend) were used following the manufacturer's protocol. Fluorescent-labeled antibodies targeting CD11b (Biolegend Cat# 101208), CD14 (Biolegend Cat# 301804), CD86 (Biolegend Cat# 305406), and CD136 (Biolegend Cat# 333632) were used to detect myeloid differentiation markers on cell surface. Flow cytometric experiments were run on BD LSR Fortessa X-20 Cell Analyzer and further analyses were performed on FlowJo X (10.0.7r2). Three separate experiments were performed for each assay and representative diagrams were shown.

Statistical analysis. Statistical significance was determined using the Student's t-test, which was performed on the GraphPad Prism software. The results were expressed as mean ± standard error of mean (SEM). Two-sided p-values less than 0.05 were considered statistically significant.

Results

Various metabolic pathways were affected in the DKI AML mouse model. The Dnmt3aR878H/WT KI mice were crossed with NrasG12D/WT KI mice to generate the DKI

mouse model (Mx1-Cre; Dnmt3aR878H/WT; NrasG12D/WT). DKI mice developed more aggressive AML than Dnmt3aR878H/WT KI mice after pIpC induction [16]. Since most leukemic cells were Gr1+ cells in the AML mice, we further analyzed the RNA-sequencing data (Supplementary Tables 1–3) of Gr1+ bone marrow cells and revealed that 1,129 genes were differentially expressed in DKI mice versus wild type mice. GO enrichment analysis showed that many enriched GO terms in biological processes in the DKI AML mice were related to metabolic pathways such as cellular amide metabolic process and ribose phosphate metabolic process (Figure 1A). Among all the enriched terms with a statistical significance ($p<0.05$), we demonstrated five terms of interest including carboxylic acid biosynthetic process, small molecule biosynthetic process, reactive oxygen species metabolic process, generation of precursor metabolites and energy, as well as nucleoside triphosphate metabolic process (Supplementary Figure S1).

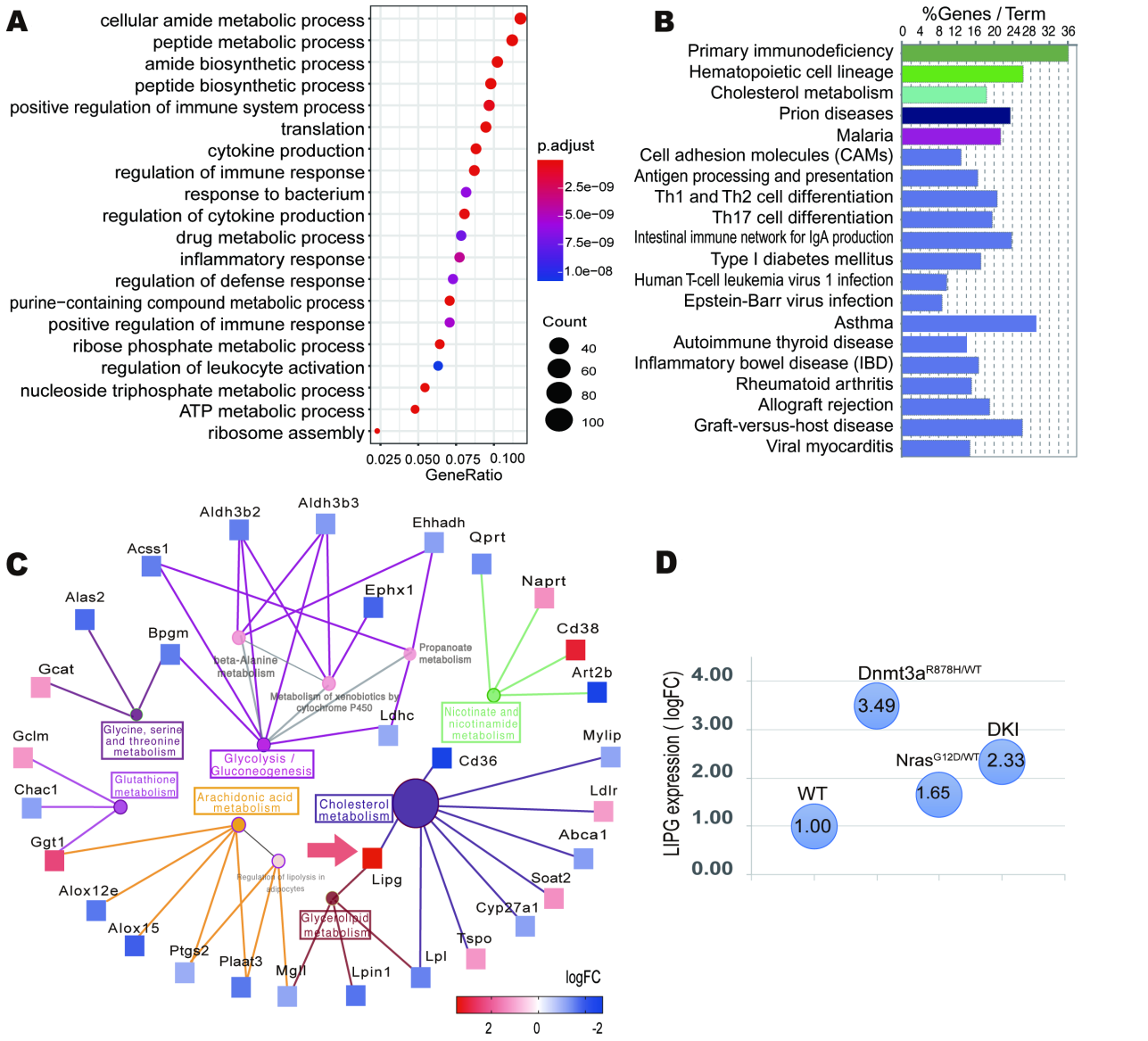

Figure 1. RNA-sequencing of AML mice with Dnmt3aR878H/WT and NrasG12D/WT mutation. A) Gene Ontology (GO) enrichment analysis of differentially expressed genes in DKI AML mice versus wild type mice. Dotplot of the top 20 enriched GO terms under the category of the biological process is exhibited. The red arrows indicate GO terms related to metabolism. B) Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis on differentially expressed genes in AML mice versus wild type mice. The bar chart of the top 20 enriched KEGG pathways. Related pathways are marked by bar color. C) The network chart of representative metabolic pathways in KEGG enrichment. Correlations between pathways and genes are also presented. D) Expression of LIPG in Gr1+ bone marrow cells of wild type (WT) mice, Dnmt3aR878H/WT mice, NrasG12D/WT mice, and DKI AML mice by RNA-sequencing. FPKM (Fragments Per Kilobase of exon model per Million mapped fragments) values were obtained and log2fold change (logFC) values are shown.

To further explore the significantly changed genes or pathways in the altered metabolic profile, we performed the KEGG analysis on the 1,129 differentially-expressed genes of the DKI AML mouse model. In the KEGG analysis, cholesterol metabolism was enriched (Figure 1B). In the network chart of differentially expressed metabolic genes enriched in the KEGG analysis, some genes associated with cholesterol metabolism were involved, including Lipg, Mylip, Ldlr, Abca1, Soat2, Cyp27a1, Tspo, Lpl, and Cd36. Among them, Lipg, Ldlr, Soat2, and Tspo were upregulated, while Mylip, Abca1, Cyp27a1, Lpl, and Cd36 were downregulated (Figure 1C). It has been reported that enhanced glycolysis could promote proliferation and resistance to drugs in AML [3, 17]. However, differential genes involved in glycolysis were mostly downregulated in the DKI AML mice (Figure 1C). Therefore, we hypothesized that enhanced lipid metabolism rather than glycolysis might contribute to the development of severe AML in mice with Dnmt3a and Nras mutation.

LIPG promotes the adaptation to stress in AML cells with DNMT3A mutation. Based on our results above, LIPG, an upregulated gene enriched in cholesterol metabolism, drove our attention. Compared to wild type mice, AML mice with Dnmt3aR878H/WT, NrasG12D/WT, or both mutations expressed a higher level of LIPG (Figure 1D). LIPG exhibits phospholipase A1 and triglyceride lipase activity, hence it can release free fatty acids and lysophosphatidylcholine from the high-density lipoprotein (HDL) [18]. Some studies have indicated that LIPG could facilitate the survival of breast cancer cells by promoting lipid precursors uptake and lipid storage [19, 20]. Hence, the role of LIPG in AML with DNMT3A mutation is worth further investigation. DNMT3A mutations are associated with poor prognosis in AML, while NPM1 mutations are considered low-risk factors and are commonly concurrent with DNMT3A mutations. Therefore, the AML cell line OCI-AML3 (OA3) harboring both DNMT3A R882C mutation and NPM1 mutation has been widely used as the *in vitro* model for studies on DNMT3A mutation-related leukemia [15, 21]. To explore the role of LIPG in AML, the CRISPR/Cas9 technique was used to knock out LIPG in OA3 cells (Figure 2A). Two groups of amplified monoclonal cells LIPGKO (KO-1 and KO-2) cells exhibited complete deletion of LIPG, and hence were used for further studies (Figures 2B, 2C).

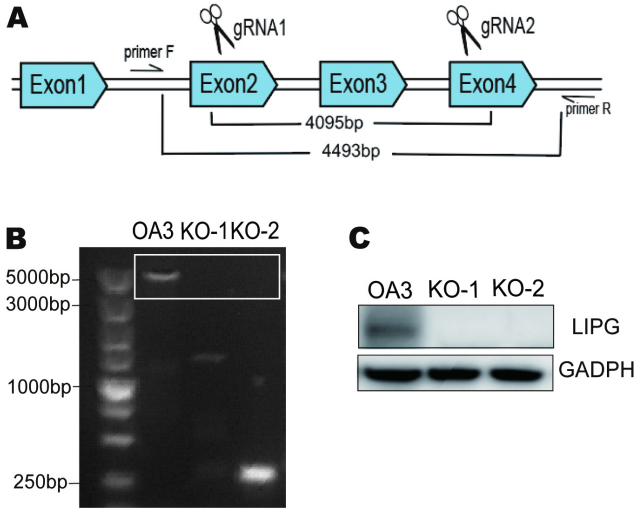

Figure 2. Gene depletion of LIPG in OCI-AML3 cells. A) Diagram of the gene-depletion of LIPG in OCI-AML3 cells using CRISPR-Cas9 technique. LIPG common exon2 and common exon4 are targeted by gRNAs. Primer F and R are used for PCR examination of knockout efficiency afterward. B, C) PCR and western blot assays showing the LIPG knockout efficiency. Control OCI-AML3 (OA3) cells and monoclonal LIPGKO cells (KO-1, KO-2) were tested.

Intracellular lipid droplets (LD) acts as a reservoir of lipids and contribute to cellular adaptability and resilience to oxidative stress [22]. OA3 cells contained abundant LD when cultured in RPMI-1640 medium supplemented with 10% FBS. LD were evidently diminished in OA3 cells after being cultured without supplemented serum for 48 h (Supplementary Figure S2). Since LIPG promotes lipid storage from HDL [18, 19], it is possible that HDL provides lipids for OA3 cells. We added 100 μg of HDL to the serum-free RPMI-1640 culture medium of OA3 cells for 48 h and noticed that the HDL group possessed comparable LD to the FBS group (Supplementary Figure S2). We concluded that OA3 cells could generate intracellular LD by feeding on supplemented serum or HDL *in vitro*. No intracellular LD were found in the LIPG knockout (LIPGKO) cell clones KO-1 and KO-2 cultured in a complete medium. Perilipin 2 (PLIN2) exists on the surface of LD of most somatic cells and modulates lipolysis by controlling the lipolysis of substrate lipids in LD. In the process of classical lipolysis and autophagic lipolysis, PLIN2 is removed from LD and rapidly degraded by the proteasome [23]. We found that PLIN2 was significantly decreased in LIPGKO cells (Figure 3A), corresponding to the absence of lipid storage when LIPG was deleted (Supplementary Figure S2).

Cancer cells bear higher endogenous oxidative stress than normal somatic cells. Thioredoxin reductase 1 (TXNRD1) is responsible for regulating oxidative stress and redox signaling [24]. By inhibiting TXNRD1, the antioxidant capacity demanded by cancer cells can be decreased while normal cells can survive [24]. High expression of TXNRD1 is correlated with a high level of LIPG in breast cancer [19]. TXNRD1 was significantly decreased in LIPGKO OA3 cells (Figure 3B), indicating impaired antioxidant capacity. Therefore, we concluded that LIPG acted as a key factor in the lipid storage of OA3 cells and could affect cellular adaptation to metabolic and oxidative stress.

LIPG contributes to the survival of OCI-AML3 cells. To investigate the influences of LIPG on cell survival, we conducted a series of experiments. Proliferation assay using cell counting kit-8 (CCK-8) indicated that the overall cell growth was evidently inhibited in LIPGKO cells compared with OA3 cells (Figure 3C). In the absence of functioning

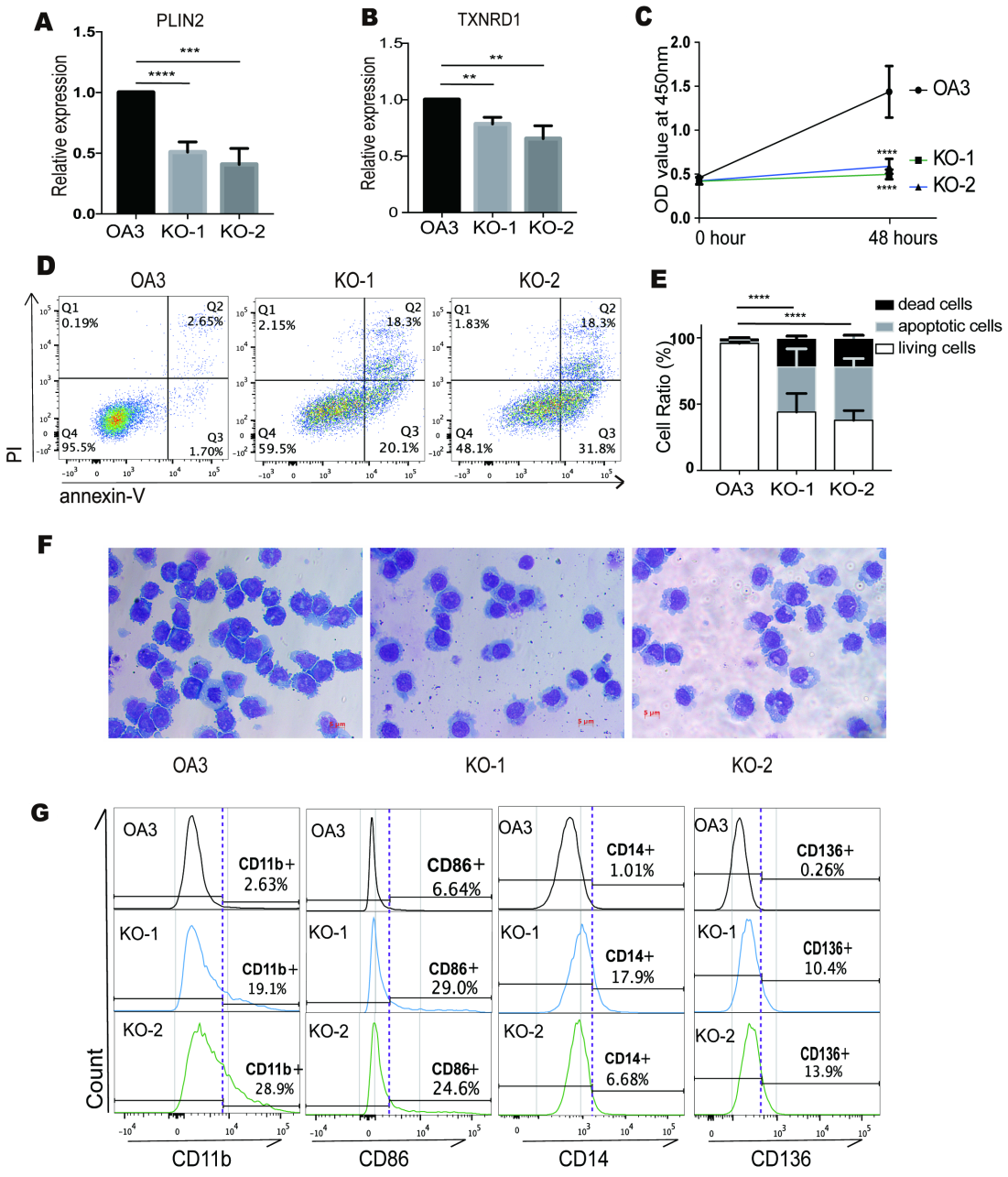

Figure 3. The effects of LIPG knockout on the survival and myeloid differentiation of AML cells. (A) qRT-PCR analysis of perilipin 2 (PLIN2) expression in OA3, KO-1, and KO-2 cells. B) qRT-PCR analysis of thioredoxin reductase 1 (TXNRD1) expression in OA3, KO-1, and KO-2 cells. Log2fold change is demonstrated and qPCR data are mean ± SEM, **p<0.01, ***p<0.001, ****p<0.0001. Each experiment was repeated at least 3 times. C) Proliferation assay of OA3, KO-1, and KO-2 cells with cell counting kit-8 (CCK-8). OD values at 450 nm were measured and compared statistically. D) Representative results of cell apoptosis assay by Annexin V/PI staining. E) Stacked bar chart displaying ratios of living, apoptotic, and dead cells in OA3, KO-1, and KO-2 cells, respectively. F) Representative Wright-Giemsa staining for the morphology of OA3, KO-1, and KO-2 cells under an optical microscope is shown. Scale bars are 5 μm. G) Expression of cell surface differentiation markers CD11b, CD14, CD86, and CD136 by flow cytometry. Gating was based on corresponding isotype control (purple dotted line). Each experiment was repeated at least 3 times. ****p<0.0001

LIPG, a larger ratio of early apoptotic and necrotic cells existed in KO-1 and KO-2 cell populations (Figures 3D, 3E). Impaired differentiation and uncontrolled clonal expansion of myeloid precursors are two major symbols of malignancy in AML [25]. Morphologically, LIPGKO OA3 cells were manifested with more characteristics of mature myeloid cells than OA3 cells (Figure 3F). Myeloid differentiation markers, such as CD11b, CD14, CD86, and CD136 were elevated in KO-1 and KO-2 cells (Figure 3G), indicating LIPG depletion can promote myeloid differentiation. OA3, Kasumi-1

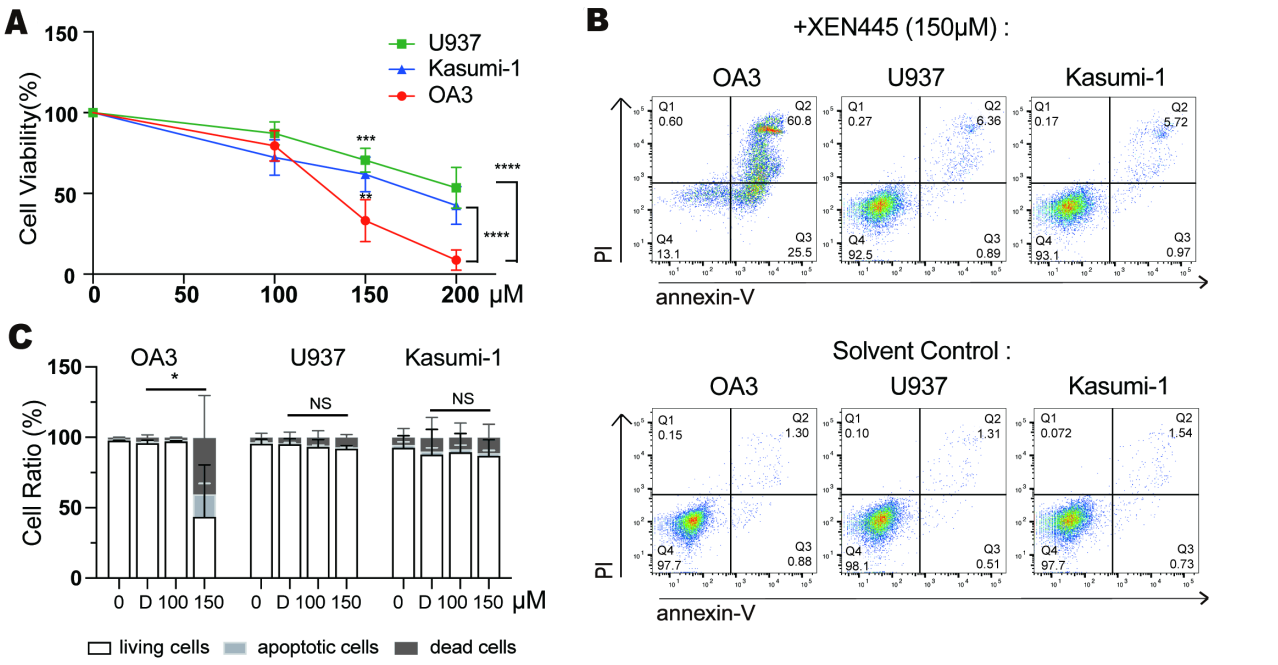

Figure 4. Effects of LIPG inhibitor on different cell lines. A) Cell counting kit-8 (CCK-8) assay of the sensitivity to XEN445 in OCI-AML3 (OA3), U937, and Kasumi-1 cells. OD values at 450 nm were measured after 48 h of incubation and relative cell viability was calculated. B) The effect of XEN445 (150 μM, above) and solvent control (DMSO, below) on apoptosis in OA3, U937, and Kasumi-1 cells. Representative diagrams are shown. C) Stacked bar chart showing ratios of living, apoptotic and dead cells after adding XEN445 (at 0, 100, 150 μM) or solvent control (DMSO) in OA3, U937, and Kasumi-1 cells. *p<0.05, ***p<0.001, ****p<0.0001. Each experiment was repeated at least 3 times.

(an AML cell line), and U937 (a histiocytic lymphoma cell line) cells were treated by XEN445, a selective LIPG inhibitor, respectively. Compared with U937 and Kasumi-1 cells, XEN445 significantly decreased the total cell viability of OA3 cells in a dose-dependent manner (Figure 4A). XEN445 also significantly promoted cell apoptosis and necrosis in OA3 cells at the concentration of 150 μM in contrast to U937 and Kasumi-1 cells (Figures 4B, 4C). In conclusion, our data suggest that LIPG contributes to the survival of AML cells by promoting lipid storage and adaptation to stress, facilitating cell proliferation, resisting apoptosis, and suppressing differentiation. OA3 cells were relatively more sensitive to LIPG inhibition than other cells without DNMT3A mutations. LIPG might be used as a potential metabolic target for AML treatment, especially for leukemic cells active in lipid metabolism such as DNMT3A mutation-related AML.

Discussion

In the carcinogenesis of solid tumors and leukemia, cancer cells maintain rapid growth under nutrient deficiency through reprogrammed metabolism [26]. Enhanced glycolysis has been reported in multiple types of cancers including AML [1, 27]. In fact, by reason of the highly heterogeneous nature of AML, metabolic profiles vary among patients [2, 28]. Certain leukemic cells were relatively inactive in glycolysis. High levels of glycolysis are associated with chemotherapy resistance, while moderate levels of glycolysis at diagnosis are predictive of poor prognosis following remission [29]. Grønningsæter et al. tested the *in vitro* antileukemic effect of seven inhibitors targeting metabolic pathways including glycolysis, glutaminolysis, the pentose phosphate pathway, and fatty acid oxidation. They found the strongest inhibitors varied in different patient-derived AML cells *in vitro*, although glycolysis inhibitor exhibited the strongest anti-leukemic effect in most AML blasts [30]. Under metabolic stress caused by glucose deficiency or anti-glycolytic drugs, some cell clones adapt by environmental selection or switching to other metabolic pathways such as lipid metabolism [2, 9]. Hence, it is worthwhile to investigate other metabolic pathways for more flexible and individualized clinical strategies. Previous studies have reported that increased consumption of fatty acids in AML is associated with drug resistance [3]. Fatty acid oxidation inhibitors such as Etomoxir and Avocatin B could sensitize leukemic cells to chemotherapeutics [7, 11]. AML cells could enhance the uptake of fatty acids for energy supply as well as biosynthesis. But related research is still lacking. Up to now, no inhibitors for lipid metabolism have been approved for AML treatment in clinic. Targeting strategies should be individualized for AML patients with varied metabolic profiles. Alternative therapeutics targeting different lipid metabolic pathways are still rare in the field of AML.

In this work, we studied metabolic reprogramming in conditional knock-in AML mice through induction of Dnmt3aR878H/WT and/or NrasG12D/WT mutation. LIPG was significantly upregulated in AML mice with Dnmt3aR878H/WT and/or NrasG12D/WT mutation. Among them, Dnmt3aR878H/WT mice expressed the highest level of LIPG. The correlation between DNMT3A mutation and LIPG remains obscure. Several molecular markers have been proven to be related to specific metabolic dysregulation. IDH1 mutant AML cells exhibit dysregulation of fatty acid metabolism [31]. It was indicated that FLT3-ITD mutation could promote serine biosynthesis in AML [32]. We previously found that DNA hypomethylation due to DNMT3A mutation could lead to the mechanistic target of rapamycin (mTOR) activation [15]. The inhibition of mTOR with rapamycin could reduce glucose uptake in AML cells, which fluctuates among different AML cell lines due to different intracellular signaling [33]. The mTOR complexes play essential roles in mammals in the aspects of lipid biosynthesis, adipogenesis, and lipid consumption [34]. mTOR could respond to anabolic signals and contribute to lipid storage by balancing other signaling pathways such as Akt and Tsc1 [35, 36]. Further researches on mTOR in lipid metabolism in AML and its correlation with LIPG were worthy to be studied. Metabonomic studies on DNMT3A mutation-related AML are still necessary, to help us clarify the influences of DNMT3A mutation on metabolism and its correlation with LIPG upregulation.

LIPG plays a critical role in lipid metabolism, as it can release free fatty acids from HDL and facilitate the transport of lipoproteins [37]. Up to now, LIPG was mostly studied in cardiovascular diseases. High LIPG levels in plasma are also associated with metabolic syndrome, obesity, and increased inflammatory markers including C-reactive protein (CRP) and interleukin 6 (IL-6) [38]. We showed that OA3 cells harboring DNMT3A R882C mutation could uptake from HDL through LIPG. LIPG contributes to form intracellular LD in AML blasts and promotes adaptation to metabolic and oxidative stress. Deletion of LIPG can inhibit cell proliferation, promote apoptosis, and myeloid differentiation in OA3 cells. Optimization of small molecule inhibitors against LIPG has been conducted. XEN445 is a selective LIPG inhibitor exhibiting good ADME and PK properties. It was reported to suppress growth and self-renewal of LIPG+ triple-negative breast cancer cells [39]. By blocking LIPG with monoclonal antibody MEDI5884, HDL level and HDL particle numbers, as well as HDL functionality, were elevated in healthy human volunteers, which were aimed to reduce the risk of cardiovascular disease [40]. Of note, we found that cells with DNMT3A mutation were much more sensitive to XEN445 than those without DNMT3A mutation. Our study indicated that LIPG might serve as a potential therapeutic target in AML, especially promising in AML blasts with an obvious inclination towards lipid metabolism or harboring DNMT3A mutation. Hence, we look forward to conducting further studies on LIPG inhibitors and other inhibitors against lipid metabolism pathways, to broaden the knowledge bank of AML treatment and facilitate personalized therapeutic decisions.

Supplementary information is available in the online version of the paper.

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Endothelial lipase promotes acute myeloid leukemia progression through metabolic reprogramming

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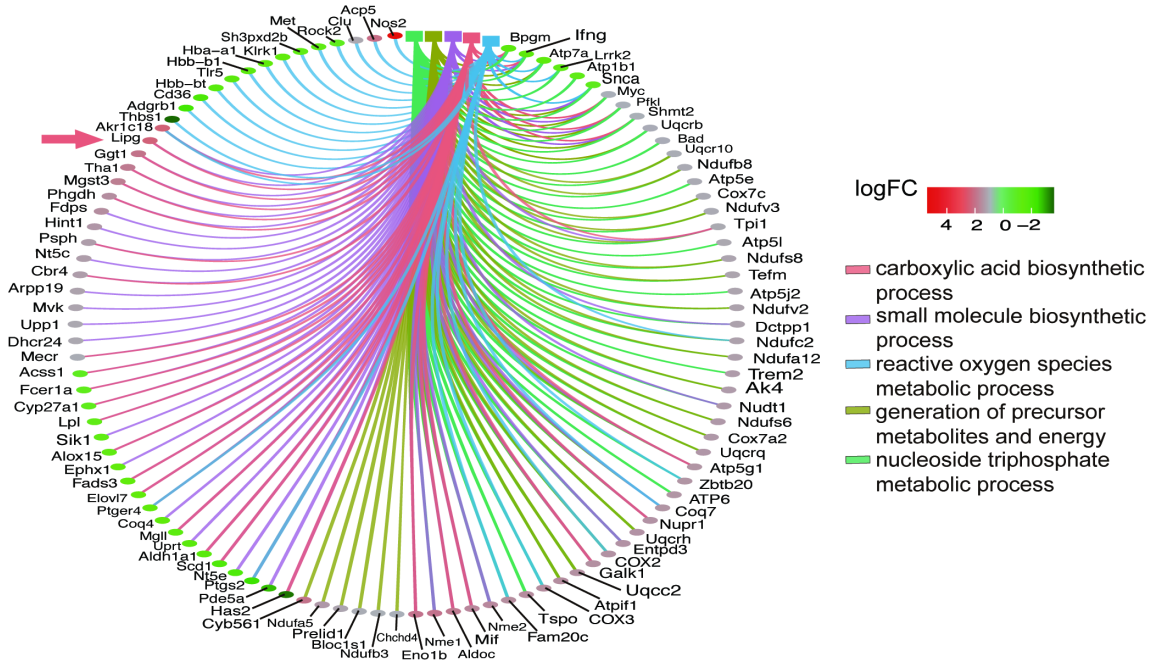
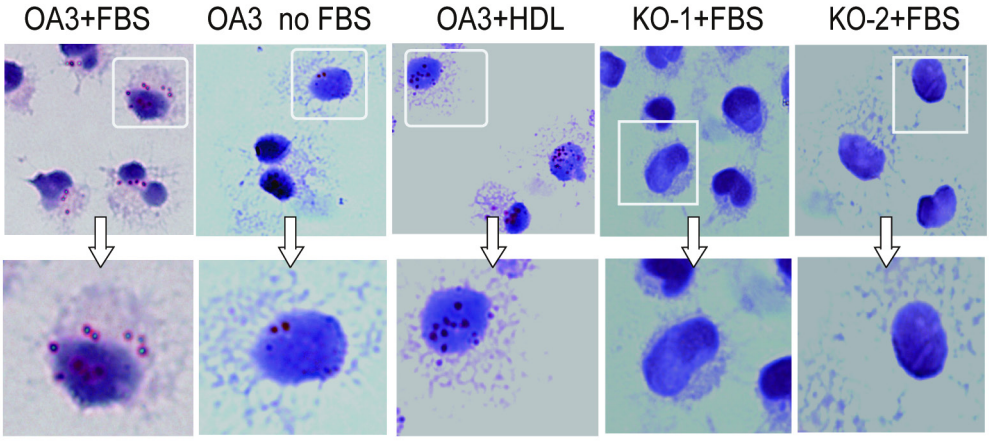
Supplementary Information



Supplementary Figure S1. Chordal graph showing metabolic genes enriched in five GO terms of interest and their expressional changes (logFC).



Supplementary Figure S2. Representative Oil RED O staining of intracellular lipid droplets in OA3, LIPG^{KO} (KO-1, KO-2) cells; lipid droplets ares shown as claret granules in cells.