Integration of Clinical and Transcriptomics Reveals Reprogramming of the Lipid Metabolism in Gastric Cancer

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Abstract

Background: Lipid metabolism has a profound impact on gastric cancer (GC) progression and is a newly targetable vulnerability for cancer therapy. Given the importance of lipids in cancer cellular processes, in this study we employed lipidomic clinical and transcriptomic data of GC to connect the variations of lipid metabolism changes.

Method: We constructed a clinical nomogram based on the lipid factors and other clinical items. Then by using multi-omics techniques, we established a lipid-related gene signature for individualized prognosis prediction in patients with GC. Moreover, a total of 1357 GC cases were then applied to evaluate the robustness of this model. WGCNA was used to identify co-expression modules and enriched genes associated with GC lipid metabolism. The role of key genes ACLY in GC was further investigated.

Results: The prognostic value of the lipidomic signature was analyzed using Cox regression model, and clinical nomogram was established. Among them, we observed overexpression of ACLY significantly increased the levels of intracellular free fatty acid and triglyceride, and activate AKT/mTOR pathway to promote cancer development.

Conclusions: In conclusion, our findings delineated a GC clinical and lipidomic signature and revealed that GC exhibited a reprogramming of lipid metabolism in association with an altered expression of lipid metabolism-associated genes. Among them, ACLY significantly promoted GC lipid metabolism and increased cancer cell proliferation, suggesting that this pathway can be targetable as a metabolic vulnerability in future GC therapy.

1. Introduction

Gastric carcinoma (GC) is commonly known as the second most cancers worldwide, which approximately accounts for 900,000 total cases and 700,000 deaths globally per annum, and the overall survival (OS) for GC patients diagnosed with metastatic still less than 1 year [1]. Hyperlipemia is a significant global health problem and regarded as a conspicuous risk factor for human cancers, specically for gastroenteric tumors, by recent statistics conducted by the International Agency for Research on Cancer[2, 3]. Lipids are classified into eight types basing on the presence of ketoacyl and isoprene groups, including fatty acids (FAs), glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketide. Epidemiological studies demonstrated that high fat diet and obesity increase the risk of GC, with obesity-persistence increase the risk of GC in a does-response manner[4]. However, the association between lipid metabolism and the pathological development of gastric cancer remained confusing, and the relationship between lipid metabolism and cancer prognosis is still not explored.

The gastrointestinal tract is an important organ for food digestion and nutrient absorption, with the supply for cellular metabolism. Lipgenesis, including de novo fatty acid synthesis and cholesterol biosynthesis, ketone body metabolism, fatty acids synthesis (FAS), fatty acids oxidation(FAO), cholesterol metabolism pre-cursor for eicosanoid synthesis, which provides cancer cells with adequate energy

supply. Cancer cells being in a nutrient-deficient environment, change their normal metabolism state for acquiring energy and building new biomass[5]. In case of nutrient deprivation, fatty acids released from lipid drops (LDs) are important energy source via mitochondria β-oxidation and Kreb's cycle. More importantly, lipid metabolism is known not only functioned in orthodox energy supply and membrane components, but also involved in numerous cellular processes, such as cell signaling, inflammation maturation, storage, turnover of proteins, cell proliferation, cytoskeleton, cell polarity, signaling molecules, post-translational modifications, angiogenesis differentiation and so on[6] [7]. Thus, lipid is gradually recognized as a prominent characteristic in a variety of cancers and attracts increasing attention.

Numerous studies have proven that lipid metabolism is a hallmark of cancer development and functions in shaping the tumor microenvironment and phenotype. The higher TC, TG, and LDL-C have been reported among patients with locally advanced, unresectable or metastatic GC compared with healthy volunteers[8]. Therefore, delineating the mechanism of lipid content in tumor microenvironment is helpful for providing fundamental insights into how lipgenesis contributes to tumor initiation and progression and predictiving index of the responses to conventional neoadjuvant therapies or immunotherapies.

With the gradually deep understanding of the extensive roles of lipid metabolism in GC pathogenesis, the specific mechanisms of cancer cells have been exploited. Our analysis integrated detailed clinical and lipidomics-transcriptomics data revealed that GC exhibited a reprogramming of lipid metabolism in association with an altered expression of lipid metabolism-associated genes. Our findings also highlighted the notion that ACLY may be a promising therapeutic target in GC and provides evidences for uncovering the link between lipodystrophy and tumor development.

2. Materials And Methods

2.1 Patients and study design

This study was designed as a retrospective cohort study, which utilized data from the First Affiliated Hospital of Wenzhou Medical University and public databases (including TCGA (https: //cancergenome.nih.gov/) and Gene Expression Omnibus (GEO) datasets (https://www.ncbi.nlm.nih.gov/geo/)). The data collection and processing protocols were approved by the institutional ethics committee (Ethics Commission of the Faculty of Medicine of the Wenzhou Medical University). All procedures were carried out in accordance to BRISQ Guidelines for reporting research on human biospecimens. These selected GC patients were histopathologically diagnosed with primary GC and then received surgical treatment with or without regular chemotherapy. All patients were followed up until death or March 2021 (end of follow-up). Patients without a pathological diagnosis of primary GC, with gastric stromal tumor subtype, who had undergone prior therapy (chemotherapy, resection prior to enrolment), did not undergo the excision or did not have complete pathology, laboratory, and follow-up data were excluded. The following demographic, clinical and pathology data were used: T stage, N stage, M stage, pathological stage, tumor history, laboratory test results (age, sex, body-mass index (BMI), TG, HDL-C, Cho, TC, CEA, creatinine). Pathologists assessed the tumor stage according to the 7th edition of the AJCC TNM staging guidelines.

Finally, a total of 458 patients were enrolled in the study. Hyperlipoidemia was defined as conform to more than one criteria : 1) Total cholesterol (TC) than or equal to 5.17mmol/l; 2) Triglyceride (TG) than or equal to 1.70mmol/l; 3) High-density lipoprotein (HDL) less or equal to 1.16mmol/l; 4) Low-density lipoprotein white (LDL) than or equal to 3.10mmol/l. Disease-free survival was defined as the time from diagnosis to tumor recurrence or occurrence of metastatic disease and overall survival as the time from diagnosis to disease-related death.

This study also utilized data from public database. We retrospectively selected GC gene expression and its clinicopathological data from the TCGA and Gene Expression Omnibus datasets. Raw microarray data Affymetrix were downloaded and normalized using the limma package. For validation, we also searched “gastric cancer” and “*Homo sapiens”* to March 2021 to select suitable chips in the GEO database. All chips with gene expression (containing at least 20 samples) from primary human gastric tumors and normal tissue were considered eligible, with no unique exclusion criteria being applied. The study contained 13 cohorts of samples from patients with GC: GSE12369, GSE13911, GSE2685, GSE26942, GSE26988, GSE29272, GSE37023, GSE54129, GSE65801, GSE66229, GSE79973, GSE84787 and TCGA STAD. Chips were summarised, together with accession numbers, in Table S1. In total, 1488 GC and 448 normal cases were acceptable for subsequent meta-analysis. The RNA-sequencing data were processed via R limma package, setting $P \leq 0.01$, fold change ≥ 1.5 as the cutoff line. The detailed working algorithm was demonstrated in Figure 1.

2.2 Construction Clinical Nomogram

The OS and PFS clinical nomograms were constructed based on the main prognostic factors to predict 1-, 3- and 5-year survival of each GC patients. A multivariable logistic regression analysis was applied to build nomogram. Each patient could sum up variable score and finally establish predictive measures of survival and relapse. The calibration curve for predicting 1-, 3- and 5-year OS and PFS indicated that the nomogram-predicted survival closely corresponded with actual survival outcomes. The survival analysis was conducted using rms, survival and survcomp package. Hazard ratios (HRs) and 95% confidence intervals (CIs) were recorded.

2.3 Establishment of the LASSO regression model and calculation of lipodystrophy risk score

We used the GSEA program to derive the enrichment scores of each lipid-metabolism Gene sets. The concrete gene lists of each lipid metabolism enrichment KEGG terms were listed in the table 2. The least absolute shrinkage and selection operator (LASSO) method which conducting with 100 iterations of 10-fold cross-validations to select the most optimal significant features and avoid overfitting, was selected to obtain the most significant genes for predicting lipid metabolism score. The features with non-zero coefficients were then selected. A lipodystrophy score was calculated for each patient via weighted by their LASSO Cox coefficients. Moreover, GSE15459, GSE26253, GSE62254 and GSE84437, which contain concrete GC patient survival information, were further employed to confirm the prognostic power of gene signature. We calculated the prognostic risk score for each patient, then K-M survival analysis were employed.

2.4 Co-expression Gene Network Based on RNA-seq Data and functional analysis

The Weighted correlation network analysis (WGCNA) was used to identify important co-expression modules and their enriched genes associated with GC lipid metabolism[9]. The proper soft threshold power (β) was chosen based on the scale-free topology criterion. The correlation between the modules and lipidscore was evaluated using Pearson's correlation coefficient analysis. Two modules with the highest average gene significance scores among all genes in the modules were selected for further study. The connectivity degree of each node of the network was calculated by STRING database and reconstructed via Cytoscape software. Gene ontology (GO) enrichment analysis was performed with the DAVID platform.

2.5 Meta-analysis

To further confirm the accuracy of conclusions, we conducted meta-analysis via Review Manager. Meta-analysis estimated the error from the heterogeneity of platform. Continuous outcomes were estimated as standard mean difference with 95 % confidence interval. Continuous outcomes were estimated as standard mean difference with 95 % confidence interval (CI).

2.6 Colony Formation and Transwell Migration Assay

Human gastric tumor cell lines BGC823 and SGC7901 were cultured in 1640 medium (Gibco company) supplemented with 10% fetal bovine serum (FBS), 100U/mL Penicilin and 100μg/mL Streptomycin. Cells were cultured in an incubator with 5% CO2 at 37°C. The number of 1×10^3 BGC823 and SGC7901 were inoculated in six-well plates and incubated at 37°C for 5 days. Cell colonies were finally fixed with 4% paraformaldehyde formaldehyde (Solarbio, Beijing, China) followed by staining with crystal violet (Sigma-Aldrich). The number of colonies was calculated. Transwell migration experiments were used to confirm the migration ability. 5×10^4 cells were added to the upper chamber placed in a 24-well plate, with serum-free medium. Meanwhile, medium containing 15% serum was added to the lower chamber. Taking cell images cell at 100× magnification.

2.7 Western blot, RT-PCR and Antibodies

An equal amount of proteins was subjected to SDS-PAGE. Proteins were transferred onto PVDF membranes, and the blots were incubated with the following different primary antibodies: Rabbit p-mTOR (S2448), p-AKT, β-actin from Cell Signaling Technology and IL6 from Proteintech. Anti-mouse and anti-rabbit antibodies were purchased from Santa-Cruz Biotechnology. All primary antibodies were confirmed to be reactive only to the targets by the manufacturer and used at 1:1000, and secondary antibodies were used at 1:5000. As for RT-PCR, the following primers were used:

ACLY forward, 5'-GACTTCGGCAGAGGTAGAGC-3', and reverse, 5'-TCAGGAGTGACCCGAGCATA-3';

GAPDH forward, 5'-TGTGGGCATCAATGGATTTGG-3' and reverse, 5'-ACACCATGTATTCCGGGTCAAT-3'.

ACACA forward, 5'-CAACAGTGGAGCAAGAATCGG-3' and reverse, 5'-TCACAATGGACAGAGTTGAGAGC-3'.

FASN forward, 5'-CCTGGCTGCCTACTACATCG-3' and reverse, 5'-CACATTTCAAAGGCCACGCA-3'.

SCD1 forward, 5'-GCGATATGCTGTGGTGCTTAATGC-3' and reverse, 5'- GGAGTGGTGGTAGTTGTGGAAGC -3'.

HMGCR forward, 5'-TGATTGACCTTTCCAGAGCAAG-3' and reverse, 5'-CTAAAATTGCCATTCCACGAGC-3'.

2.8 Quantification of free fatty acid and cholesterol

We prepared chloroform/methanol (2:1) for extracting lipids. The levels of free fatty acid and cholesterol were determined with EnzyChromTM free fatty acid and cholesterol kits (Bioassay Systems, Hayward, CA, USA).

2.9 Immunohistochemistry

20 GC specimens were collected, of which 10 hyperlipemia and 10 non- hyperlipemia GC tissues. Two researchers evaluated the staining results independently and scored the intensities of immunostaining as: 0 (negative), 1 (weakly positive), 2 (moderately positive) and 3 (strongly positive).

2.10 Statistical analysis

Statistical analysis was conducted using R software (v. 3.0.1; http:// www.Rproject.org), SPSS (v. 21.0.0.0; https://www.ibm.com), and GraphPad Prism 6. Kaplan-Meier curves and log-rank tests were used to predict OS and PFS in relation to lipid metabolism. Univariate and multivariate Cox regression analyses were performed to calculate corresponding hazard ratios (HRs) and 95% confidence interval (CI). All statistical tests were two-sided and $P < 0.05$ was considered statistically significant.

3. Results

3.1 Association of lipid biomarkers with clinicopathological feature

According to our selection criteria, a total of 458 GC patients were included in this retrospective study. One hundred and three (22.5%) patients were female, and 355 (77.5%) were male; the median age was 64.3 (range, 29–87) years. About 38.9% of the patients only received surgery, while the others received both regular adjuvant postoperative therapy and surgery. The median follow-up for OS and PFS was 40.67 and 37.21 months respectively. Hyperlipemia is conferred as meeting one of the following items: the serum total TC, TG and LDL-C are increased and HDL-C is decreased. In our study, the proportions of patients with high- TC, TG, LDL-C and HDL-C were 26.64%, 29.04%, 25.33% and 27.51%, respectively. Totally, there were 288 patients diagnosed with hyperlipemia and 170 patients with non- hyperlipemia. Baseline clinicopathological characteristics were summarized in Table 1.

The results of univariate Cox hazards analysis was presented in figure 2 and figure S1. The univariate analysis demonstrated that HDL, TC and LDL were significant associated with PFS ($P < 0.05$). Although some univariate Cox hazard results of lipid factors did not reach statistical significance, we saw a clear trend and called the result promising and worth further studies. An OS and PFS nomogram were constructed to predict 1-, 3- and 5-year overall survival (Figure 2B) and PFS (Figure S1B) of GC patients. Total scores were summations of each variable based on the intersection of the vertical line. As shown in Figure 2B and figure S1B, TC and LDL contributed the most risk points, whereas the other clinical information contributed much less. By sum of the total points from all variables combined with the location at the total point, we could obtain the probabilities of survival outcomes of 1-year, 3-year, and 5-year. In addition, decision curve analysis showed that both the predictive accuracy of prognostic nomograms for OS and PFS (Figure 2C and figure S1C).

3.2 Identification of Lipid Metabolism Signature in patients with GC.

Gene Set Enrichment Analysis (GSEA) is a computational method that defined set of genes shows statistically significant differences between two biological states[10]. The significantly enriched lipid-related pathways included unsaturated fatty acids, fatty acid metabolism, steroid biosynthesis, ether lipid metabolism and glycosphingolipid biosynthesis lacto and neolacto serie. A total of 133 lipid-relative genes were significantly enriched in the pathways related to lipid metabolism and gastric cancer (Table 2). LASSO Cox regression was performed to identify the most important features in terms of predicting the survival of GC patients (Figures 3A). By forcing the sum of the absolute value of the regression coefficients to be less than a fixed value, certain coefficients were reduced to exactly zero, and the most powerful prognostic features (ACLY, ABCG4, ABCG1, FTO, ABCA2, IGFBP7) were identified with relative regression coefficients. The prognostic risk score model was established with the following formula: lipid score = expression level of ACLY × 0.47 + expression level of ABCG4 × 0.133 +expression level of ABCG1 × 0.2238+expression level of FTO× 0.274-expression level of ABCA2 × 0.385+expression level of IGFBP7 × 0.210. All patients were divided into the high- and the low-lipid group using the median score as the cutoff line (Figure 3B-3D). K-M survival analysis showed that high risk group had significantly poorer OS than that of low (log-rank $P <0.001$). In addition, following the univariate and multivariate analyses, the lipid signature also showed to be an independent prognostic factor in the GC cohorts (95%HR1.78-2.12, $P < 0.001$; 95%HR1.65-1.92 $P < 0.001$).

3.3 Evaluation of the prognostic lipid metabolism signature in external validation cohorts

To verify the accuracy of prognostic model identified by TCGA were also important in additional GC cases, we further selected eligible cohorts of GC cases from the GEO database (GSE15459, GSE26253, GSE62254 and GSE84437). As result, a total of 1357 GC cases were applied to evaluate the robustness of our model. Consistent with the results in the train cohort, the four survival analysis all showed that high lipid group had a worse prognosis than those with low one. The distribution of lipid score, and survival information of patients were analyzed and showed in Figure 4A-4C. In brief, these external

validation outcomes combination with prior studies demonstrated that our lipid signature were powerful enough to precisely discriminate high lipid score of GC patients.

3.4 Functional Annotation and WGNCA of GC Patients

To further identify the underlying biological characteristics in the lipid metabolism signature, WGCNA was performed, and the correlation of lipid score and module membership were analyzed. The soft threshold selection is shown in Figure 5A. The yellow module had a significant p-value with both lipid signature (Figure 5B). The association between module membership and gene significance for each gene in the brown module is shown in Figure 5C. To better annotate the module function, we singled out the 20 central genes in the co-expression network whose MM > 0.8. As shown in the figure 5D, ACLY is the hub gene in the GC lipid regulation.

3.5 ACLY markedly elevated expression in the GC tissues

Genome data from TCGA suggested that ACLY gene is amplified in mostly GC cases (figure 6A). To further determine the ACLY mRNA expression in GC tissues, we examined the open GEO datasets which contain both GC specimens and normal specimens (Additional Table1). In total, 1488 GC and 448 normal cases were enrolled in the subsequent meta analyses. Notably, the results showed that the ACLY mRNA expression exhibited a significantly increasing trend in group of GC specimens compared with normal specimens ($Z = 4.34$; $p < 0.0001$, figure 6B). These meta-analyze combination with prior outcomes mainfested that ACLY mRNA expression was significantly enhanced in GC tissues.

To evaluate the clinical significance of ACLY overexpression, we analyzed its protein expression by immunohistochemistry (IHC) in 30 GC patients. As shown in the figure 6C, GC patients with high ACLY expression were endowed with advanced pathological stage than those with low ACLY expression in cohorts. Essentially the same result was seen with HPA GC cohort (Figure 6D). These results robustly demonstrate that ACLY was an independent prognostic predictor of poor survival in patients with GC.

3.6 ACLY increased the expression levels of fatty acid synthesis enzymes and AKT/mTOR signaling

To study the role of ACLY in the lipid metabolism of GC cells, we designed to measure the changes of lipid content in gastric cancer cells with relative higher and lower ACLY expression. As shown in the figure 7A-7B, overexpression of ACLY significantly increased the levels of intracellular free fatty acid and triglyceride, while knockdown of ACLY markedly reduced the levels of those lipids. We further investigated the expression levels of key molecules involved in fatty acid metabolism (ACACA, FASN, SCD1, HMGCR) in GC tissue when ACLY was knocked-down or over-expressed. As shown in the figure 7C-7D, the expression of ACLY resulted in significantly change expression levels of those lipogenic enzymes. Thus, we inferred that ACLY increased de novo fatty acid synthesis and cholesterol biosynthesis in GC tissues. To provide further support, the expression levels of ACLY and lipogenic enzymes were determined in 380 GC tissue samples from TCGA. Spearman rank correlation analysis indicated significantly positive

correlations between the expression levels of ACLY and lipogenic enzymes of ACACA (r=0.345, p <0.0001), FASN (r=0.413, <0.0001), SCD1 (r=0.300, <0.0001) and HMGCR (r=0.286, <0.0001) (Figure 7E).

It is consensus viewed that lipids are a broad church of hydrophobic biomolecules that participate in a wide array of metabolic pathways, and can influence cancer cell biology via a range of multiple oncogenic signaling pathways. Considering the mTOR pathway is a master regulator of cell growth and metabolism in response to nutrient signals, particularly lipid. A key example is the well-defined influence of PI3K-mTOR on the cell biology by phosphorylating the membrane lipid phosphatidylinositol (4,5)-bisphosphate to phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P3), which activates multiple oncogenic signaling pathways, such as AKT signaling, during tumor progression [11]. Considering that AKT/mTOR pathway has been well established to play a central role in the regulation of cell lipid metabolism[12, 13], we hypothesized that ACLY overexpression may activate AKT/mTOR pathway to promote cancer development. As expected, ectopic expression of ACLY was sufficient to robustly promote GC cell migration and transwell compared with control cells (Figure 6F and 6G). ACLY knockdown significantly decreased the phosphorylation levels of AKT and mTOR, whereas in inverse, indicating that ACLY activates AKT/ mTOR signaling in GC cells (figure 7H-7I). Thus, we may come up with an assumption that ACLY increased GC progression by activating AKT/mTOR signaling.

4. Discussion

Hyperlipemia and its related complications are popular health problems, and the global prevalence has estimated 3.9 million [14]. Emerging evidences have demonstrated that lipid function in the development and malignant progression of various cancers. Higher LD accumulation was regarded as a new hallmark of cancer cells, such as colorectal cancer, gastric cancer, breast and prostate cancers, hepatocellular carcinoma, renal cell carcinoma and glioblastoma, which is increasingly recognized as one of the characteristics of aggressive cancer[15]. It has been proven that high numbers of lipid droplets containing triglycerides and cholesterol are stored in lipid droplets were correlated with a poorer prognosis and shorter disease-free survival for many types of cancers. Moreover cancer stem cells also have been discovered containg higher amounts of lipid droplets than their differentiated counterparts. Given that lipids regulate very diverse cellular processes and influence a wide range of GC tumorigenic steps in cancer development, progression and metastasis, there is substantial clinical interest in developing therapies to target lipid metabolism.

As an important organ of digestive system, stomach plays an important role in lipid absorption and metabolism, which was proven both in vitro and in vivo. Endoscopic histology studies found that a large number of lipid drops existed in the GC tissues, which is considered to be a hallmark of GC leading to enhanced lipid synthesis[16, 17]. A higher lipid content and higher rate of de novo lipogenesis has been observed as well in gastric cancer and adjacent pericarcinomatous tissue. Our results were in good agreement with recent data describing enhanced lipogenesis in GCs. We also have demonstrated that the level of lipids in the blood can also distinguished advanced patients from GC individuals.

The differences of lipid metabolism in GC have received renewed interest since altered lipid homeostasis has been identified as a contributing factor to GC progression. However, its underlying mechanisms remains not completely understood. In this context, we integrated clinical and lipidomics-transcriptomics data revealed that GC exhibits a reprogramming of fatty acid metabolism with an altered lipid level and lipid metabolism-associated gene model. The possible association mechanism between these two risk factors was firstly described in this paper and focused on shedding light on the candidate signature genes and biological events occurring during GC progression. There have been many studies come up to explain for this phenomenon. Tumor cells acquire diet-derived FAs from the blood, and subsequently use for production of more energy for cancer progression. In case of cancer cell nutrient deprivation, fatty acids released from LDs are used for energy production via mitochondria β-oxidation and Kreb's cycle[18]. Moreover, the extracellular FAs also provide a flexible compensatory mechanism for cancer cells under conditions of metabolic stress. Even more, increased lipid metabolism, such as β-oxidation rate for metastatic cells, is inclined to metabolism[19]. Moreover, lipolysis bounding to the luminal surface for remodeling cell membranes, which potentially be supplied to enhance saturation of membrane. Lipid membrane may tend to function in making cancer cells less susceptible to free radicals and penetration of chemotherapeutic agents[20] .

ACLY is the upstream enzyme linking carbohydrate to lipid metabolism, which generates acetyl-CoA from citrate for oxaloacetate and acetyl on the cytosolic side, as well as the acetylation of protein substrates including histones [21]. ACLY is regradly considered as a bridge connecting glycometabolism and lipid metabolism. ACLY is repeatedly proven overexpressed in many types of cancers and tumor progression [22], including osteosarcoma, cervical cancer, prostate cancer and lung cancer, hepatic, colorectal cancers. Consistently, our data show that ACLY promoted the de novo fatty acid synthesis of GC cells through up-regulation of the lipogenic enzymes of ACACA1, FASN and SCD1, which further support the oncogenic role for dysregulated lipogenic enzymes in the promotion of cancer progression. ACLY is an important enzyme in the cholesterol biosynthetic pathway, which regulates multi-task of lipid metabolism. First and most important, ACLY catalyzes the conversion of citric acid to oxaloacetate and AcCoA, which promotes key lipid metabolism enzymes [23, 24]. ACLY is an upstream of the HMGCR important in the cholesterol biosynthetic pathway. ACLY regulates the expression of sterol transporter ATP-binding cassette transporter G5/8(ABCG5/8), FABP7, oxaloacetate and AcCoA [25], which catalyzes the final step in reverse cholesterol transport. Meanwhile, ACLY gene promoter contains a sterol response element (SRE) whose expression is regulated by sterol regulatory element binding protein-1α (SREBP-1α) [22, 26]. Besides, ACLY is involved in the chemotherapeutic efficacy of GC. For example, ACLY is an intermediate of the AMPK pathway, which plays a key role in the treatment of chemoresistant of breast cancer[27] .ACLY also plays a significant role in the AKT signaling pathway, promoting the survival of drug-resistant colorectal cancer cells [28]. ACLY inhibitors have widespread clinical efficacy in treating dyslipidemia and other cardiovascular disorders as monotherapy, or combination therapy with other lipid-modulating drugs. Mounting studies have shown that various ACLY inhibitors have pharmacological effects specifically in the reduction of the level of non-HDL-C, TG and insulin, as well as increasing plasma β-hydroxybutyrate level. In addition to down-regulation the level of lipids, it also displays tumor

suppressive effects,which attenuates aerobic glycolysis of tumor cells in vitro, and also reduces tumor growth and induces differentiation in vivo [29, 30]. In this study, we found that ACLY robustly promote GC cell lipid metabolism and mTOR pathway, which further facilitate tumor cell migration and transwell (Figure 7). It has been proven that using ACLY inhibitors could weaken the promoting effect of lipid on the proliferation and viability of tumor cells [31].

Hyperactive of mTORC1 signaling is a major cause of human tumors, and it has been widely described as a potential target for cancer therapy. Some rapamycin analogs (rapalogs), everolimus and temsirolimus, have been approved for some advanced carcinomas. Therefore, studying the regulation of mTORC1 is of considerable biological and clinical importance. Lipid is an essential nutrient for cancer progression, and lipid has been proven rapidly activates mTORC1. Considering that Akt/mTOR pathway has been well established to play a central role in the regulation of cell lipid metabolism, we interred that blocking ACLY which lessen the lipid synthesis may alleviate tumor cell resistance to TKI therapy and enhance the antitumor efficiency of TKI. ACLY monitors nuclear receptors to promote transcription of ABC family transporter ABCB1/ABCG2, involved in the development of multi-drug resistance[32]. Mehdizadeh et al. reported that the change of fatty acids distribution in gastrointestinal cancer cells is associated with side effects of conventional chemotherapy. LDs accumulation might impair drug-induced apoptosis as well as immunogenic cell death, resulting in the chemotherapy resistance of cancer cells.

The strengths of this research include exploring and verifying the relationship between lipid metabolism and the prognosis and survival of GC from the perspective of epidemiology and molecular mechanisms. The study explored the possible mechanism by which lipodystrophy leads to the malignant progression of GC, and partially verified its mechanism by in vitro experiments. Nevertheless, our understanding of the precise role of lipid metabolism in GC is still very limited. Firstly, this study was a monocenter prospective research, some selection, calculation bias and deviations were unavoidable. The outcomes should be validated by multicenter prospective information. Moreover, some confounding factors were not assessed in this research, such as drinking, occupation, the history of smoking, and multiple cancers, which may confuse the causal relationship between lipodystrophy and GC prognosis. Also if possible, further experiments could be performance in vivo and in vitro to verify these results.

In conclusion, the current study reveals that lipid metabolism is a risk factor for a poor GC prognosis and promises to be a potential prognostic indicator. ACLY affects mainly tumors development through the mTOR pathway, resulting in a poor GC prognosis for patients. Further study demonstrated that the combined treatment of inhibition of ACLY and mTOR pathway is expected to become a new treatment for GC patients with lipodystriphy, which will be verified in future experiments.

Declarations

Ethics approval and consent to participate

This study was retrospective study and approved by the institutional research board of the First Affiliated Hospital of Wenzhou Medical University.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the TCGA, ICGC and GEO database and the First Affiliated Hospital of Wenzhou Medical University.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Weiyang Cai, Yilun Xu and Renpin Chen conceived and designed the experiments. Yanyan Li, Jungang Zhao and Shengwei Chen performed in data collection. Weiyang Cai, Yanyan Li analyzed the data. Jungang Zhao and Yanyan Li wrote the manuscript.

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Tables

Table1 Clinicopathological characteristics of gastric cancer patients grouped by lipid index.

Characteristics	TC		TG		HDL		LDL	
	<5.1	≥5.1	<1.7	≥1.7	≥1.42	<1.42	<3.1	≥3.1
Sex		0.159		**<0.001**		**0.003**		**0.003**
Male	64	39	76	89	85	18	63	40
Female	272	83	266	27	240	115	269	86
T stage		**0.001**		**0.004**		**<0.001**		**0.001**
T1	52	38	55	35	74	16	50	40
T2	47	19	50	16	53	13	49	17
T3	44	14	41	17	46	12	45	13
T4	193	51	196	48	152	92	188	56
N stage		**0.016**		**0.001**		0.463		**0.013**
N0	127	63	125	65	139	51	125	65
N1	55	20	63	12	55	20	54	21
N2	154	39	154	39	131	62	153	40
M stage		0.159		**0.041**		0.578		0.725
M0	306	116	310	112	298	124	305	117
M1	30	6	32	4	27	9	27	9
Stage		**0.005**		**0.002**		**<0.001**		**0.043**
1	75	43	74	44	98	20	73	45
2	87	22	50	16	52	14	47	19
3	187	51	186	52	148	90	185	53
4	30	6	32	4	27	9	27	9
Chemotherapy		0.899		**<0.001**		**0.041**		0.280
No	130	48	122	56	136	42	124	54
Yes	206	74	220	60	189	91	208	72
BMI		**0.034**		0.099		0.889		0.064
<18.5	35	5	35	6	28	12	34	6
18.5-25	301	117	307	110	297	121	298	120

Table2 The specific markers for Lipid-related pathways

KEGG	Gene
Unsaturated fatty acids	ACOT2, ACOT7, ACOT4, HACD2, ACAA1, HADHA, FADS1, ACOX1, HSD17B12, ELOVL2, YOD1, PECR, BAAT, ELOVL5, SCD, ACOT1, ELOVL6, ACOX3, HACD1, FADS2
Fatty acid metabolism	TECR, ACAA2, ECI2, ADH1A, ADH1B, ADH1C, CPT1C, ADH4, ADH5, ADH6, ADH7, CPT1A, CPT1B, CPT2, CYP4A11, ECI1, ECHS1, EHHADH, ALDH2, ACSL1, ACSL3, ACSL4, ALDH1B1, ALDH9A1, ALDH3A2, ACSL6, GCDH, CYP4A22, ACAA1, HADHA, HADHB, HADH, ACADL, ACADM, ACADS, ACADSB, ACADVL, ACAT1, ACAT2, ALDH7A1, ACOX1, ACSL5, ACOX
Steroid biosynthesis	CEL, EBP, CYP27B1, CYP51A1, DHCR7, DHCR24, FDFT1, LIPA, LSS, NSDHL, HSD17B7, MSMO1, SC5D, SOAT1, SQLE, TM7SF2, SOAT2,
Ether lipid metabolism	PLA2G4B, PLA2G4E, ENPP6, LPCAT4, PLA2G2D, PLA2G2E, PLA2G2C, PAFAH1B1, PLA2G3, PAFAH1B2, PAFAH1B3, PAFAH2, ENPP2, PLA2G1B, PLA2G2A, PLA2G5, PLD1, PLD2, LPCAT2, CHPT1, PLA2G2F, PLA2G7, LPCAT1, PLA2G12A, PLA2G6, PLA2G10, PLA2G12B, AGPS, PLPP1, PLPP2, PLPP3
Glycosphingolipid biosynthesis lacto and neolacto serie	B3GALT5, B3GNT3, ST3GAL6, B3GNT2, FUT9, B4GAT1, FUT1, FUT2, FUT3, FUT4, FUT5, FUT6, FUT7, B4GALT1, ABO, ST3GAL4, ST3GAL3, ST8SIA1, B3GN4, B3GNT5, B4GALT4, B2GAL3, B4GALT2, B3GALT1

Figures

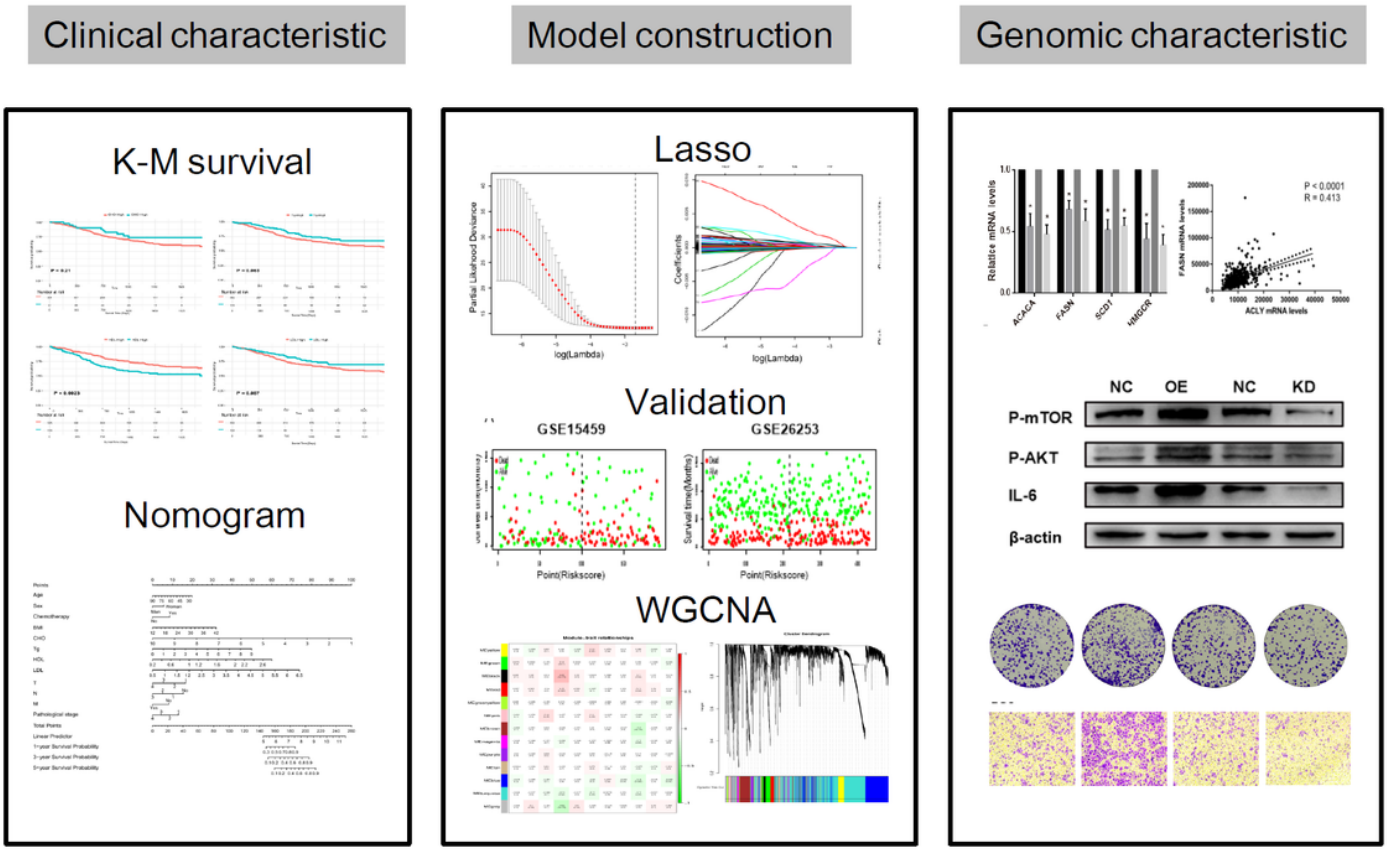

Figure 1

Flow chart of the experimental design and main process.

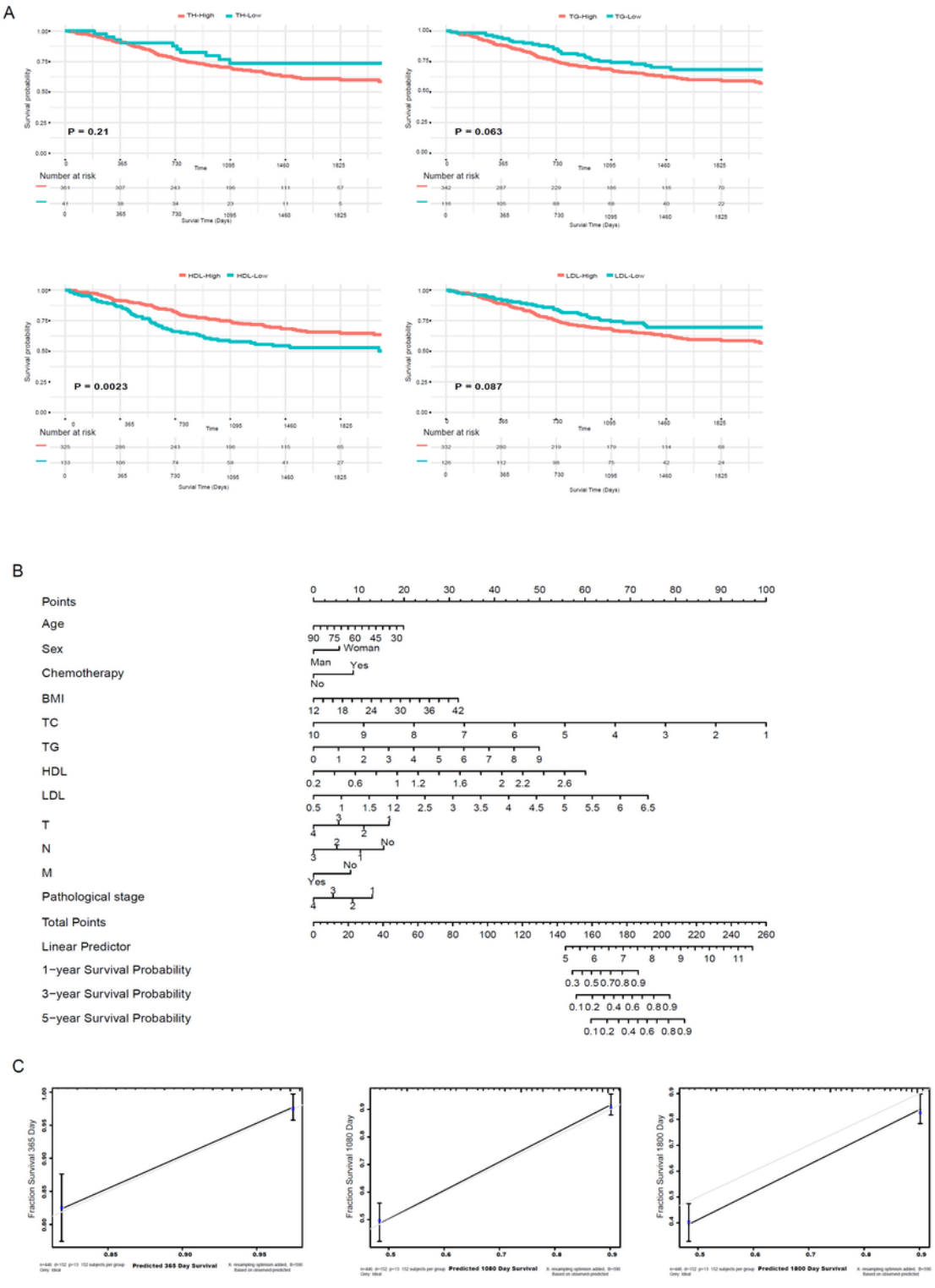

Figure 2

Kaplan-Meier curves for GC patients stratified by clinical lipid index. (A) Kaplan-Meier analysis of overall survival (OS) of Cholesterol, Triglyceride, HDL and LDL;(B)Nomogram developed by integrating lipid index and other clinical pathological parameters for predicting 1-, 3-, 5-year survival of hyperlipemia GC patients; (C)Calibration curve for risk of 1-, 3-, 5-year survival of hyperlipemia GC patients ;

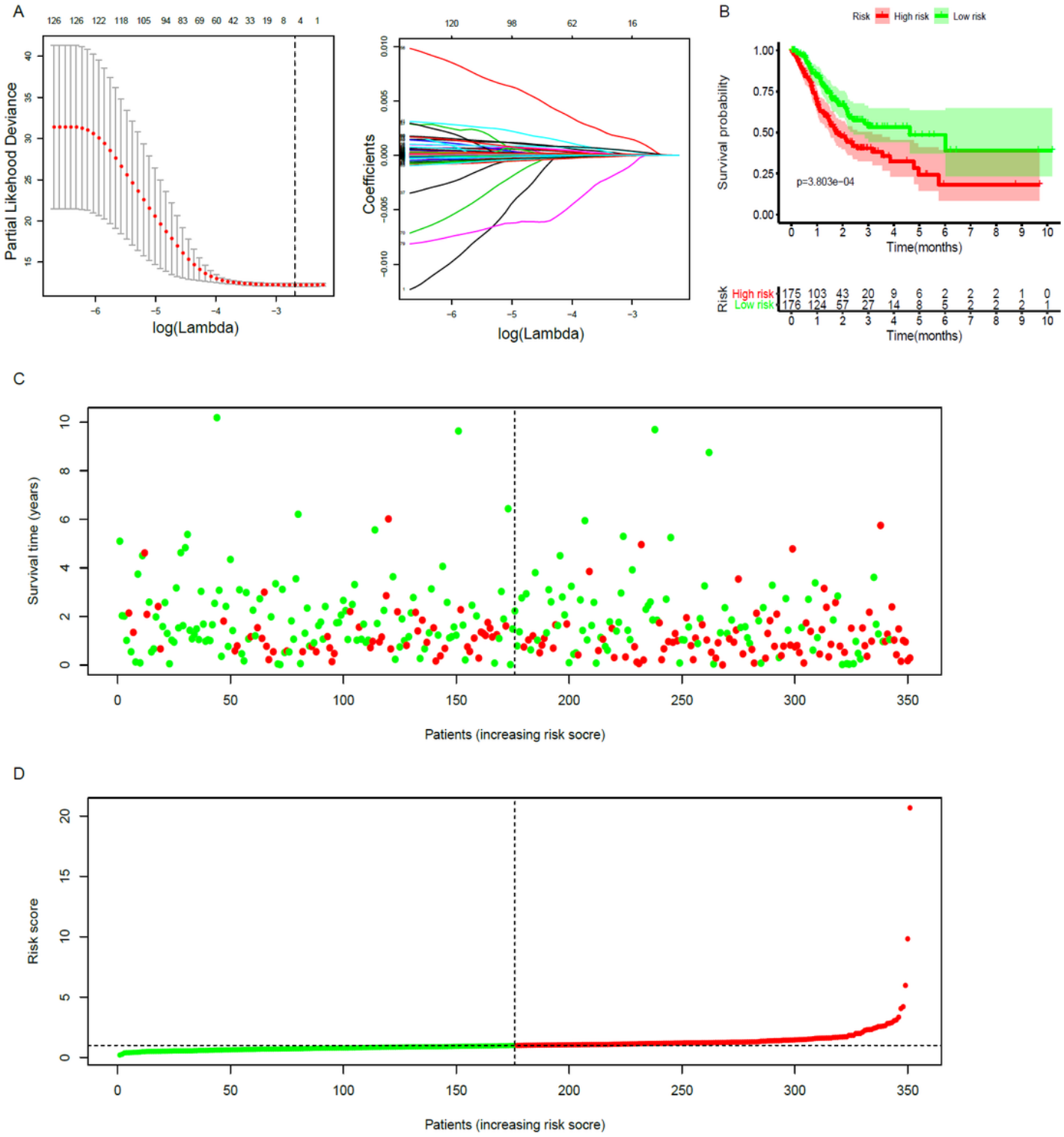

Figure 3

The distribution of gene lipid score in the TCGA GC cohort. (A) Feature selection with LASSO binary logistic regression model. The left part: The longitudinal solid line represents the partial likelihood deviation ±standard error and the longitudinal dotted line indicates that the best parameter is selected according to the minimum value (left) and 1-SE (right). Lambda is the tuning parameter. The right part: y axis represents Coefficients. Each curve in the graph corresponds to the value of each characteristic

regression coefficient varying with the log(Lambda) value. (B) K-M survival curve of the low- and high-lipid score for TCGA GC patients; (C-D)The distributions of the lipid score and survival status for each GC patients based on lipid score;

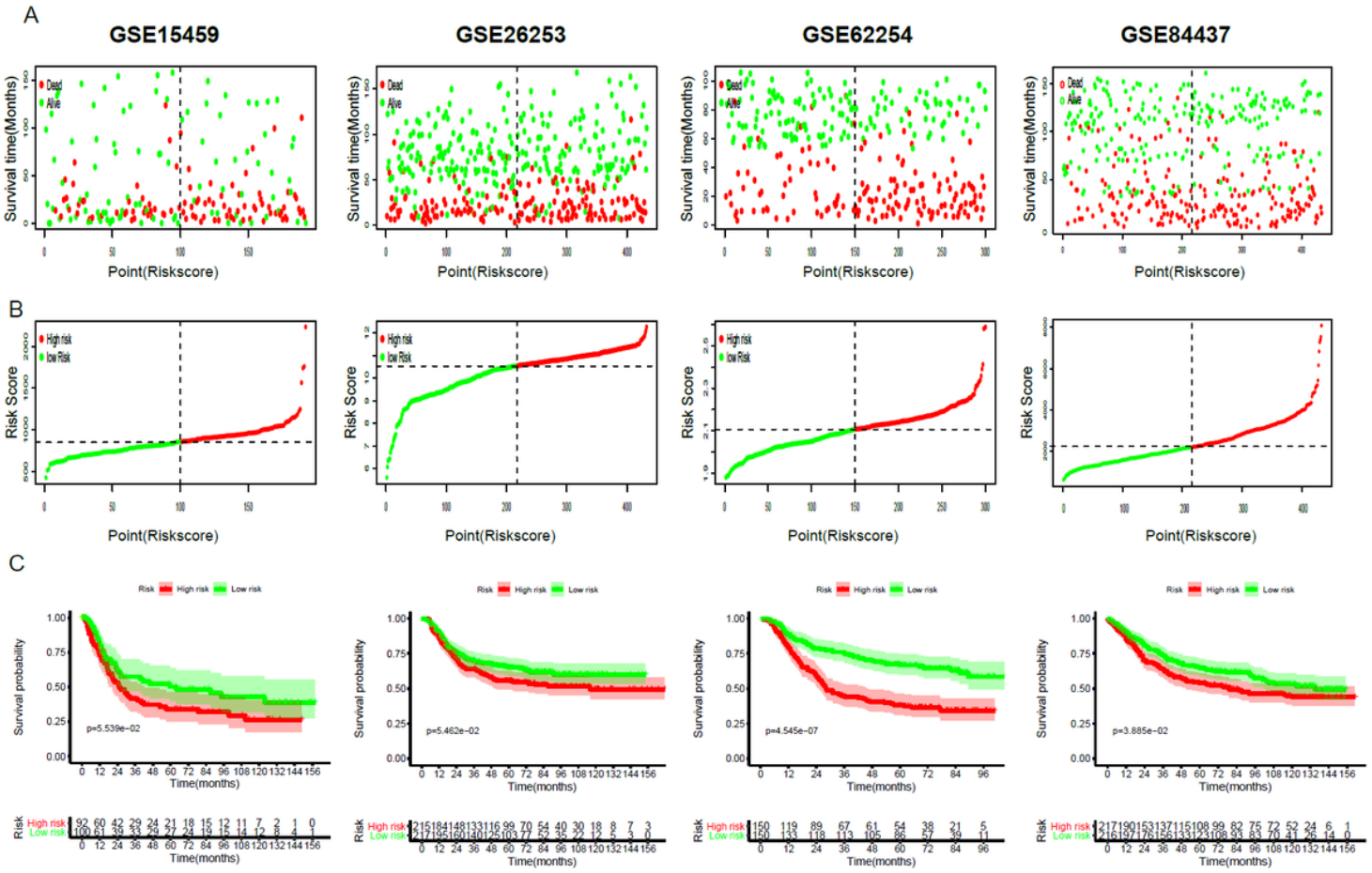

Figure 4

Validation and comparsion of the lipodystrophy signature in GSE15459, GSE26253, GSE62254 and GSE84437 validation cohort. (A)Patient survival status and time distributed by lipid score for each validation cohort; (B) the distribution of lipid score for each validation cohort; (C)K -M survival curve of the lipid score for the OS time of each validation cohort. The colors from green to red indicate the expression level from low to high. The dotted line indicates the individual inflection point of the risk score curve, by which the patients were categorized into low-risk and high-risk groups.

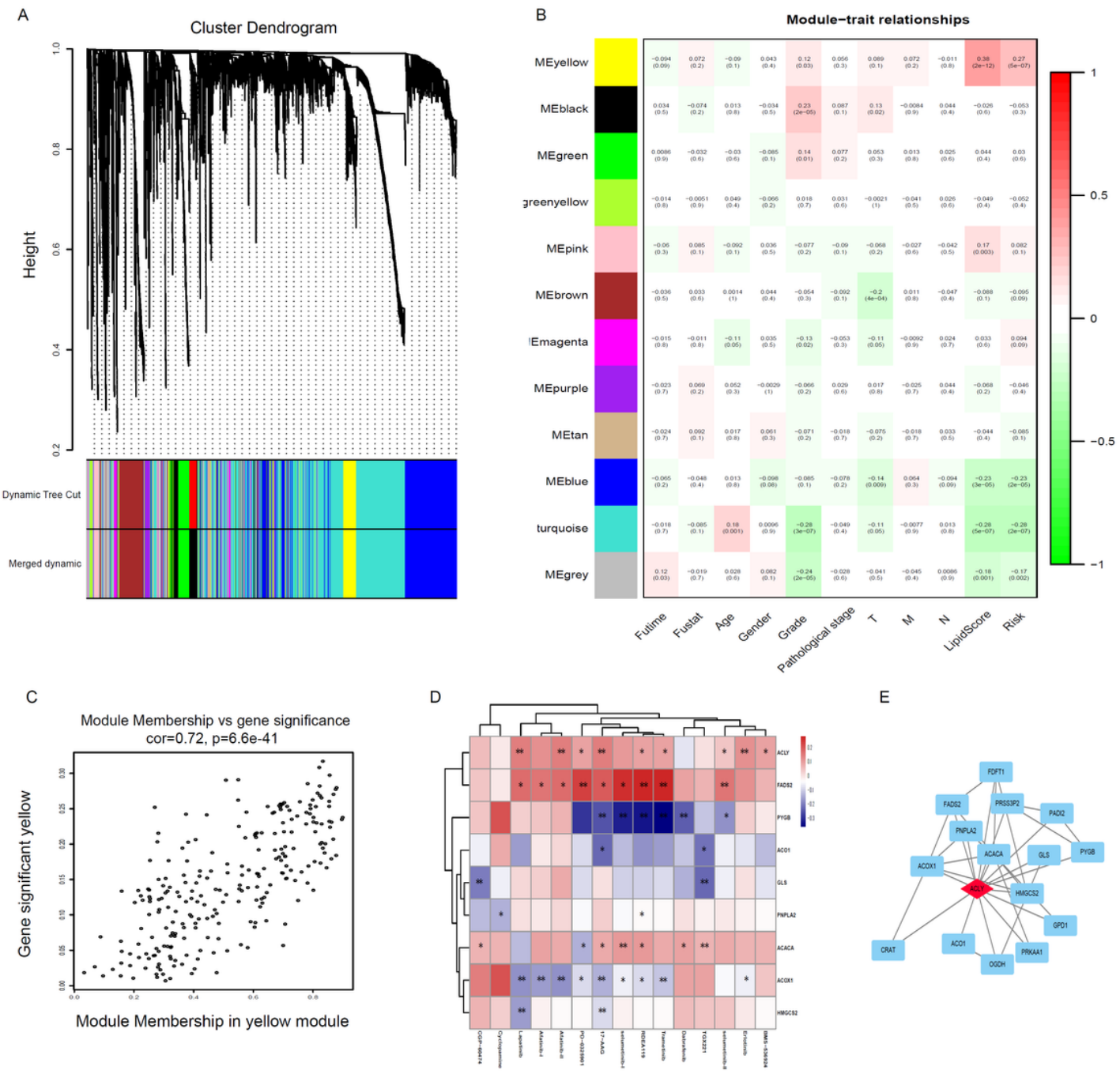

Figure 5

Weight gene co-expression network analysis (WGCNA) identified lipid -related modules eigengenes. (A) Hierarchical clustering dendrogram of identified gene in modules of GC. (B) Heat map to show the correlation between module eigengenes and the clinical traits; The right color scale indicates the association. Red, positive associations; green, negative associations. The left color scale is corresponding to each module. (C) The correlation plot of module membership and gene significance in the yellow module; (D) Construction of the PPI network for top differentially expressed mRNAs in the yellow module.

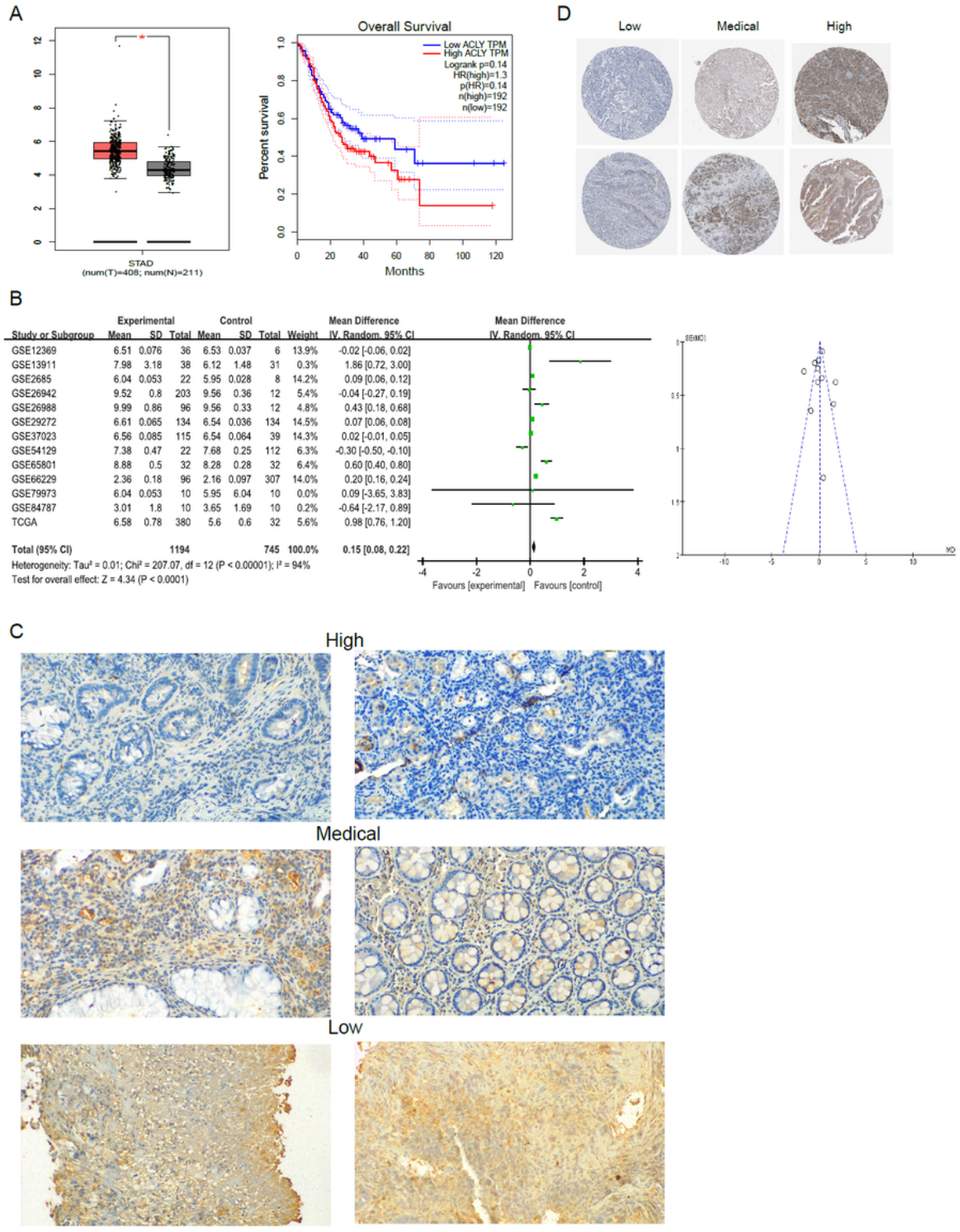

Figure 6

Expression and survival analysis of ACLY in GC. (A) The mRNA expression of ACLY in normal and GC tissues in the TCGA GC dataset. K-M OS curves based on the expression levels of ACLY; (B) Meta-analyze verified ACLY mRNA expression in 13 datasets; (C) ACLY immunostaining of representative images of GC patients with different IHC scores; (D) T ACLY immunostaining of representative images of GC patients in HPA database;

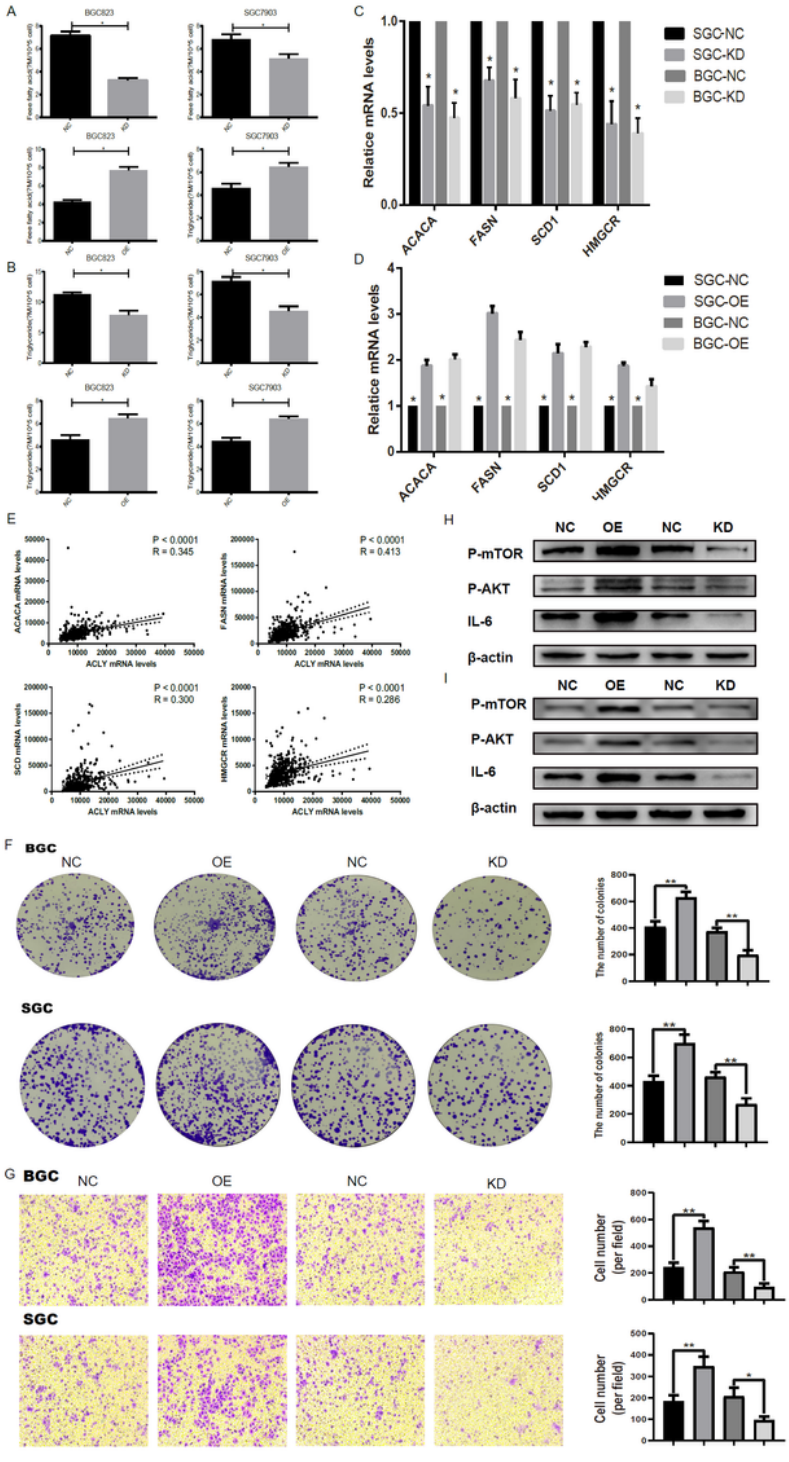

Figure 7

ACLY increased the expression levels of fatty acid synthesis enzymes and AKT/mTOR signaling. (A) Intracellular levels of free fatty acid in BGC823 and SGC7901 with ACLY knocked-down or over-expressed; (B) Intracellular levels of triglyceride in BGC823 and SGC7901 with ACLY knocked-down or over-expressed; (C) Quantitative RT-PCR analysis for mRNA levels of lipogenic enzymes of ACACA, FASN, SCD1 and HMGCR in BGC823 and SGC7901 with ACLY knocked-down;(D) Quantitative RT-PCR analysis

for mRNA levels of lipogenic enzymes of ACACA, FASN, SCD1 and HMGCR in BGC823 and SGC7901 with ACLY over-expression; (E) Spearman correlation analysis of the relationship between the mRNA expression levels of ACLY and lipogenic enzymes; (F) Colony formation assay in BGC823 and SGC7901 cells with different treatment as indicated; (G) Transwell matrigel invasion assay for invasion ability in BGC823 and SGC7901 cells with different treatment as indicated;(H) Knowdown ACLY inhibited mTOR signaling in GC cells. (I) ACLY promoted mTOR signaling in GC cells. BGC823 and SGC7901 expressing ectopic ACLY or vector were analyzed for mTOR signaling by immunoblotting;

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