ACAT2 may be a novel predictive biomarker and treated target in lung adenocarcinoma

ZHONGCHAO WANG[1], ZHUGEN CAO[2], and ZHAOXIA DAI[3]

[1]Dalian Medical University
[2]Suqian First People's Hospital
[3]The Second Hospital of Dalian Medical University

February 25, 2023

Abstract

Background: Acyl-coenzyme A cholesterol acyltransferase (ACAT) is a membrane-binding enzyme, which localizes in the endoplasmic reticulum. ACAT2 can promote the progression of colon cancer, but its efficacy in lung adenocarcinoma(LUAD) is still not sure. Method: ACAT2 expression analysis was performed by TIMER2.0 database. GEPIA database was utilized to analyse co-relations between expression of ACAT2 and pathological stage of tumor. Kaplan-Meier analysis was analyzed its potential in clinical prognosis. CancerSEA database analysed correlations between expression of ACAT2 and functional status of different tumor displayed as a heatmap. The molecule interaction network analysis performed by the STRING tool. Results: ACAT2 was upregulated in patients with LUAD, and high expression of ACAT2 had a poor DFS and OS. Cox regression analysis indicated that the poor outcomes might be related to the tumour stage, nodal stage, distant metastatic stage. ACAT2 participated in biological process of cell cycle, DNA repair, DNA damage, proliferation. Enrichment pathway analysis showed four ACAT2-correlated genes, ACOX1, EHHADH, OXCT1, DLAT. Conclusion: ACAT2 might be a novel predictive biomarker and treated target.

1 INTRODUCTION

In China, lung malignant tumor is one of the most usual and lethal cancers. Non small cell lung cancer (NSCLC) accounts for about 85%,which is the most common type[1]. The most prevalent pathological type is lung adenocarcinoma (LUAD)[2]. The primary treatment approaches include surgery, immunotherapy, chemotherapy, radiotherapy and targeted therapy, lung cancer that is detected early-on always has a better prognosis after surgery. However, most lung cancer patients, who lost the opportunity of surgical treatment, are diagnosed at the middle or late period. The overall survival rate of 5-year is frustratingly low (˜18.1%) [3]. Lung cancer is asymptomatic at an earlier preclinical stage. Thus, early diagnosis is difficult. Therefore, it is highly warranted to explore a new non-invasive diagnostic molecular biomarker for detecting the early stage lung carcinoma, prognosis evaluation and an individualized treatment plan.

Acyl-coenzyme A cholesterol acyltransferase (ACAT) that localizes in the endoplasmic reticulum, is a membrane-binding enzyme. The formation of bycholesteryl ester is obtained by ACAT catalyzed long chain fatty acyl-CoA and cholesterol[4]. Excess cholesterol esters are stored in the cell as lipid droplets. ACAT1 and ACAT2 are isoforms of the ACAT family. ACAT1 is ubiquitously expressed in normal human tissues, which generates steryl esters that are integrated into cellular lipid droplets[5]. ACAT2 offers cholesteryl esters for lipoprotein assemblies, which is principally expressed in fetal liver and intestine[6]. In clear cell renal cell carcinoma, decreased expression of ACAT2 indicates poor prognosis[7]. A recent study show that ACAT2 is overexpressed in tissues and cell lines of colon cancer(CRC). High expression of ACAT2 is associated with rapidly malignant progression and poor patient outcomes. In vitro, downregulation of ACAT2 inhibited

the growth of CRC cells[8]. Nevertheless, the relationships between ACAT2 expression and the prognosis of LUAD and their molecular mechanisms are not clear.

In our research, we aim to examine the expression of ACAT2 in pan-cancer and LUAD tissues, and to explore the prognostic significance of ACAT2 in LUAD patients.

2 MATERIALS AND METHODS

2.1 Expression level of ACAT2 in pan-cancer and LUAD patients

To explore the expression of ACAT2 mRNA between normal tissues and tumor in pan-cancer and LUAD tissues, we used Tumor Immune Estimation Resourc(TIMER2.0 database) (https://cistrome.shinyapps.io/timer/) and Xiantao bioinformatics tool (https://www.xiantao.love/products). The University of Alabama at Birmingham Cancer data analysis Portal(UALCAN database) was analysed ACAT2 total protein level in LUAD patients and normal tissues(http://ualcan.path.uab.edu/) . Immunohistochemistry analysis was performed by the human protein atlas (https://www.proteinatlas.org/).

2.2 Prognostic significance of ACAT2 in LUAD patients

In order to explore the prognostic significance of ACAT2, correlations between expression of ACAT2 and functional status of tumor were analysed by Gene Expression Profiling Interactive Analysis(GEPIA database) (http://gepia.cancer-pku.cn/). To compare the differences of disease free survival (DFS) and overall survival (OS), Kaplan-Meier (K-M) curves were plotted. Independent prognostic factor analysis was performed by Xiantao bioinformatics toolbox.

2.3 Correlations between ACAT2 expression and tumor functional status

CancerSEA database analysed correlations between expression of ACAT2 and functional status of different tumor displayed as a heatmap (http://biocc.hrbmu.edu.cn/CancerSEA/). There were fourteen functional status in the database, namely,apoptosis, angiogenesis, DNA damage, invasion, cell cycle, EMT, differentiation, DNA repair, proliferation, inflammation, hypoxia, metastasis, quiescence and stemness. ACAT2 expression profiles at single-cell levels of LUAD were displayed by the T-SNE diagram.

2.4 ACAT2-related genes enrichment analysis

The molecule interaction network analysis performed by the STRING tool (https://cn.string-db.org/). Pearson's coefficient was utilized to analyse ACAT2 and the selected genes by the Xiantao bioinformatics tool. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment assay of ACAT2 related genes were carried out in the Xiantao bioinformatics tool.

2.5 Statistical analysis

In TIMER2.0 database, the Wilcoxon test was used for the statistical significance. For the comparison of tumor and normal samples, the ANOVA method was used in GEPIA2 database. Correlations between ACAT2 and its related genes analysis used by Spearman's correlation coefcients. OS and DFS analysis in LUAD patients used by Kaplan-Meier curve and the log-rank test. Statistically signifcant differences were $P<0.05$.

3 Results

3.1 Abnormal expression with ACAT2 in pan-cancer and LUAD patients

In our study, to compare with the ACAT2 mRNA expression levels in tumor and normal tissues, TIMER2.0 database was performed (Figure 1A). The ACAT2 mRNA levels were overexpressed in the following cancer. For instance, bladder urothelial carcinoma (BLCA), esophageal squamous cell carcinoma (ESCA), breast invasive carcinoma (BRCA), kidney renal clear cell carcinoma (KIRC), cholangiocarcinoma (CHOL), lung

adenocarcinoma (LUAD), head and neck squamous cell carcinoma-human papillomavirus positive (HNSC-HPVpos), lung squamous cell carcinoma (LUSC), head and neck squamous cell carcinoma-human papillomavirus negative (HNSC-HPVneg), kidney renal papillary cell carcinoma (KIRP), uterine corpus endometrial carcinoma (UCEC), thyroid carcinoma (THCA). Conversely, ACAT2 manifested low expression in stomach adenocarcinoma (STAD), head and neck squamous cell carcinoma (HNSC), prostate adenocarcinoma (PRAD), liver hepatocellular carcinoma (LIHC).

To further explore the differential expression of ACAT2 in LUAD patients, the expression of ACAT2 mRNA was performed by Xiantao bioinformatics toolbox. The expression level of ACAT2 in LUAD tissues was higher than normal tissues (Figure 1B,1C). To determine protein expression levels, we used CPTAC database to analysis the ACAT2 protein expression (Figure 1D).

To test whether ACAT2 was also upregulated in tumor tissues, immunohistochemistry analysis was performed in normal and tumour tissues. Compared to normal adjacent tissues, tumour tissues generally had significantly higher expression level for ACAT2 (Figure 1E).

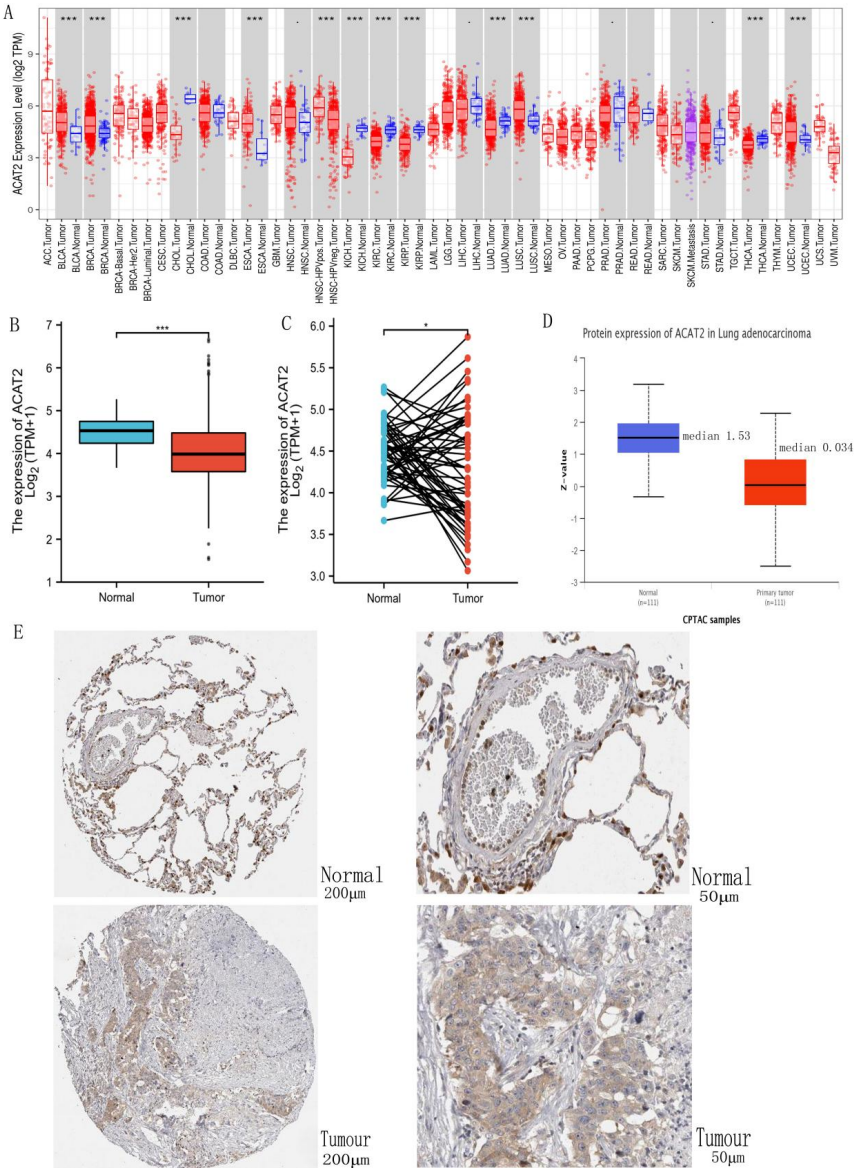

FIGURE 1 Abnormal ACAT2 expression in pan-cancer and LUAD patients.

A. TIMER2 database was performed to detect ACAT2 mRNA level. B. The unpaired samples examined ACAT2 mRNA expression level in LUAD and normal group. C. The paired samples examined ACAT2 mRNA expression level in LUAD and normal group. D. Total protein level of ACAT2 in normal tissue and LUAD performed by CPTAC. E. Immunohistochemistry analysis was performed in normal and tumour tissues. P-value: ns p¿0.05, * p< 0.05,** p<0.01, *** p<0.001.

3.2 Prognostic analysis of ACAT2 in LUAD patients

We used the GEPIA2 tool to explore the relationship between the expression of ACAT2 and tumor pathological stage. (Figure 2A). Furthermore, compared with the high expression group, Kaplan-Meier analysis also demonstrated that disease free survival (DFS) and overall survival (OS) of the low expression lever were better. (Figure 2B, 2C). Cox proportional hazards model was used for univariate and multivariate survival analysis. As a result, univariate and multivariate Cox regression analysis indicated that the poor outcomes may be related to the tumour stage, nodal stage, distant metastatic stage. (Figure 2D,2E). The predictive effect of ROC profile exceeded other clinical factors (sex, age, KPS, pathological stage, and so on). (Figure 2F). Model for predicting sensitivity and specificity of patients was assessed by time-dependent ROC curves. During 1-, 3-, and 5-year periods, OS of the area under the ROC curve was 0.605, 0.582, and 0.523,respectively. (Figure 2G).

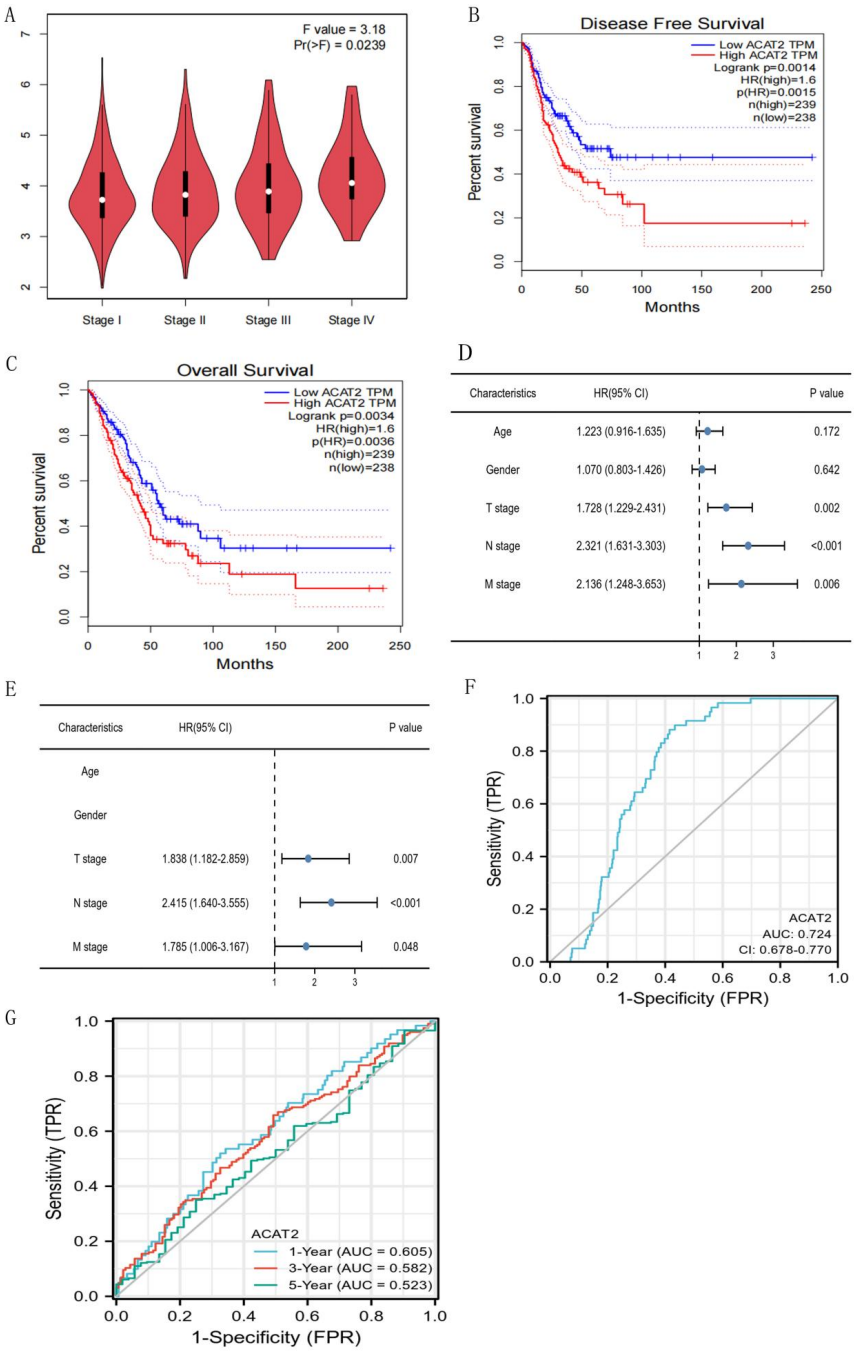

FIGURE 2 Prognostic analysis of ACAT2 in LUAD patients.

GEPIA2 showed relations between expression of ACAT2 and pathological stage of tumor. B. DFS survival curves between high expression ACAT2 and low expression ACAT2 groups. C. OS survival curves between high expression ACAT2 and low expression ACAT2 groups. D. Independent prognostic factors were performed by univariate COX regression analysis. E. Independent prognostic factors were performed by multivariable COX regression analysis. F. The ACAT2 ROC curve. G. OS of the area under the ROC curve

was analysed to predict survival at 1-, 3-, and 5-year.

3.3 Correlation between expression of ACAT2 and functional status of different tumor

Single cell sequencing technology can be used to study cancer heterogeneity. CancerSEA website at the single cell level was utilized to detect relations between ACAT2 expression and its correlation with the tumor functional status in different cancers. There was highly correlated between expression of ACAT2 and fourteen tumor functional statuses in the heatmap (Figure 3A). ACAT2 was significantly and positively correlated with cell cycle (r=0.39, P=0.00), DNA repair (r=0.37, P=0.00), DNA damage (r=0.26, P=0.00), and proliferation(r=0.24, P=0.01) (Figure 3B). Simultaneously, there were strong negative relations between ACAT2 and quiescence (r=-0.33, P=0.00), differentiation (r=-0.28, P=0.00), angiogenesis (r=-0.27, P=0.00), metastasis(r=-0.22, P=0.01), inflammation (r=-0.21, P=0.02) (Figure 3C). The T-SNE diagram for LUAD at single-cell levels showed ACAT2 expression profiles. (Figure 3D). The above evidence revealed that ACAT2 might play a vital part in the biological processes in the progression of cancer.

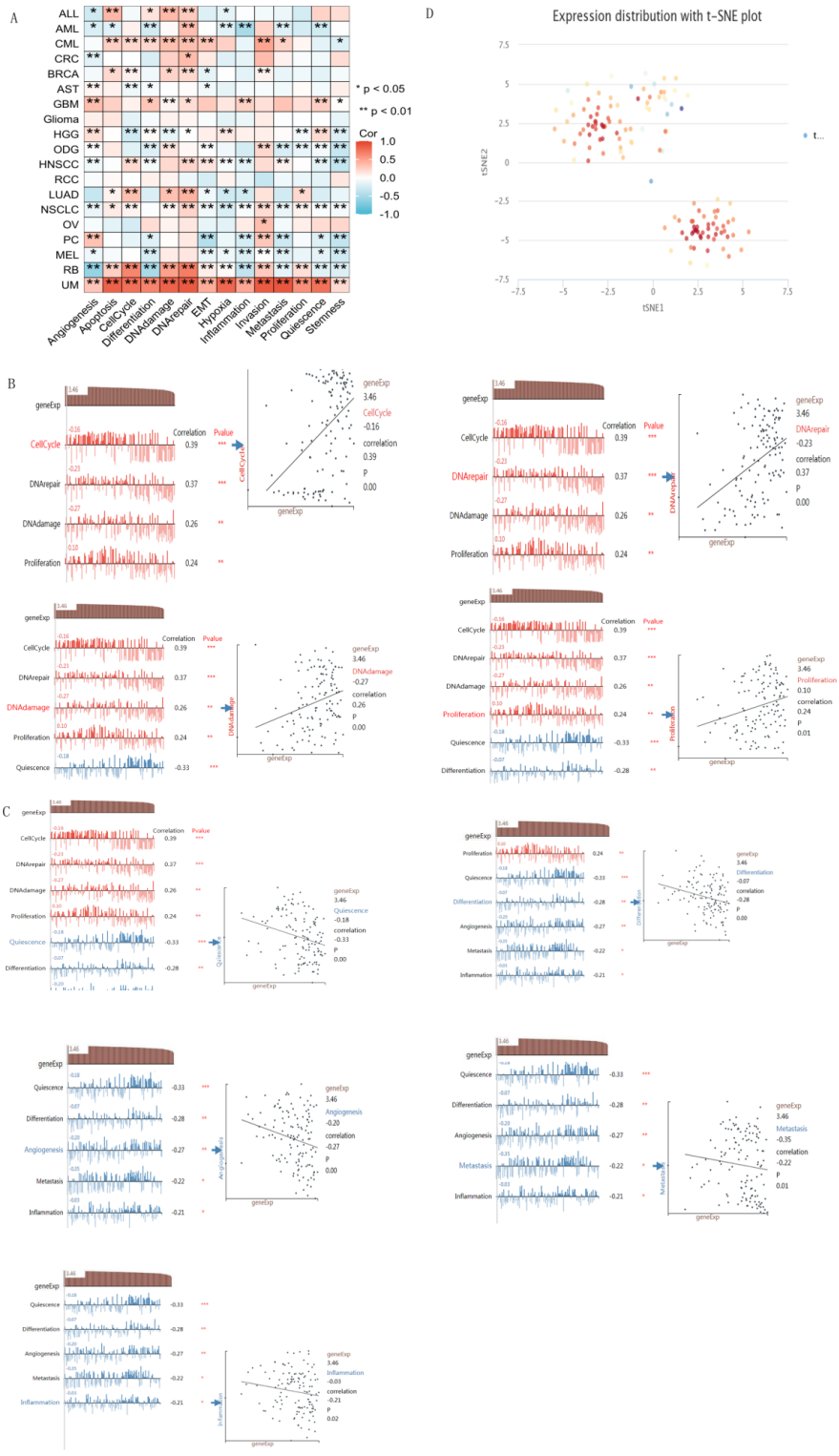

FIGURE 3 Correlation between ACAT2 expression and different tumor functional status

A. CancerSEA database displayed the correlation between functional status of different tumor and expression

of ACAT2. B. and C. Different functional status of ACAT2-related expression. D. The T-SNE diagram for LUAD at single-cell levels showed ACAT2 expression profiles. P value: ns. $p>0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

3.4 Analysis of ACAT2-correlated co-expression network and enrichment pathway

To further elucidate ACAT2 molecular mechanism, ACAT2-related molecule networks were constructed by the STRING tool. ACAT2-binding molecules were acquired, as shown in (Figure 4 A). The GEPIA2 database was analyzed top four ACAT2-correlated genes. The expression of ACAT2 was strong positive correlated with acyl-CoA oxidase 1 (ACOX1, R=0.11, P=0.0024), enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase (EHHADH, R=0.12, P=0.00041), 3-oxoacid CoA-transferase 1 (OXCT1, R=0.21, P=2e-09), dihydrolipoamide S-acetyltransferase (DLAT, R=0.19, P=6.8e-08) (Figure 4 B). The GO and KEGG pathway analysis exposed ACAT2-related biological process and cellular components (Figure 4 C). These biological process mainly contained DNA replication,chromosome segregation, regulation of mitotic cell cycle phase transition, chromosomal and centromeric region, condensed chromosome, chromosome, activity of catalytic, activity of DNA helicase and DNA-dependent ATPase, cell cycle, proteasome.

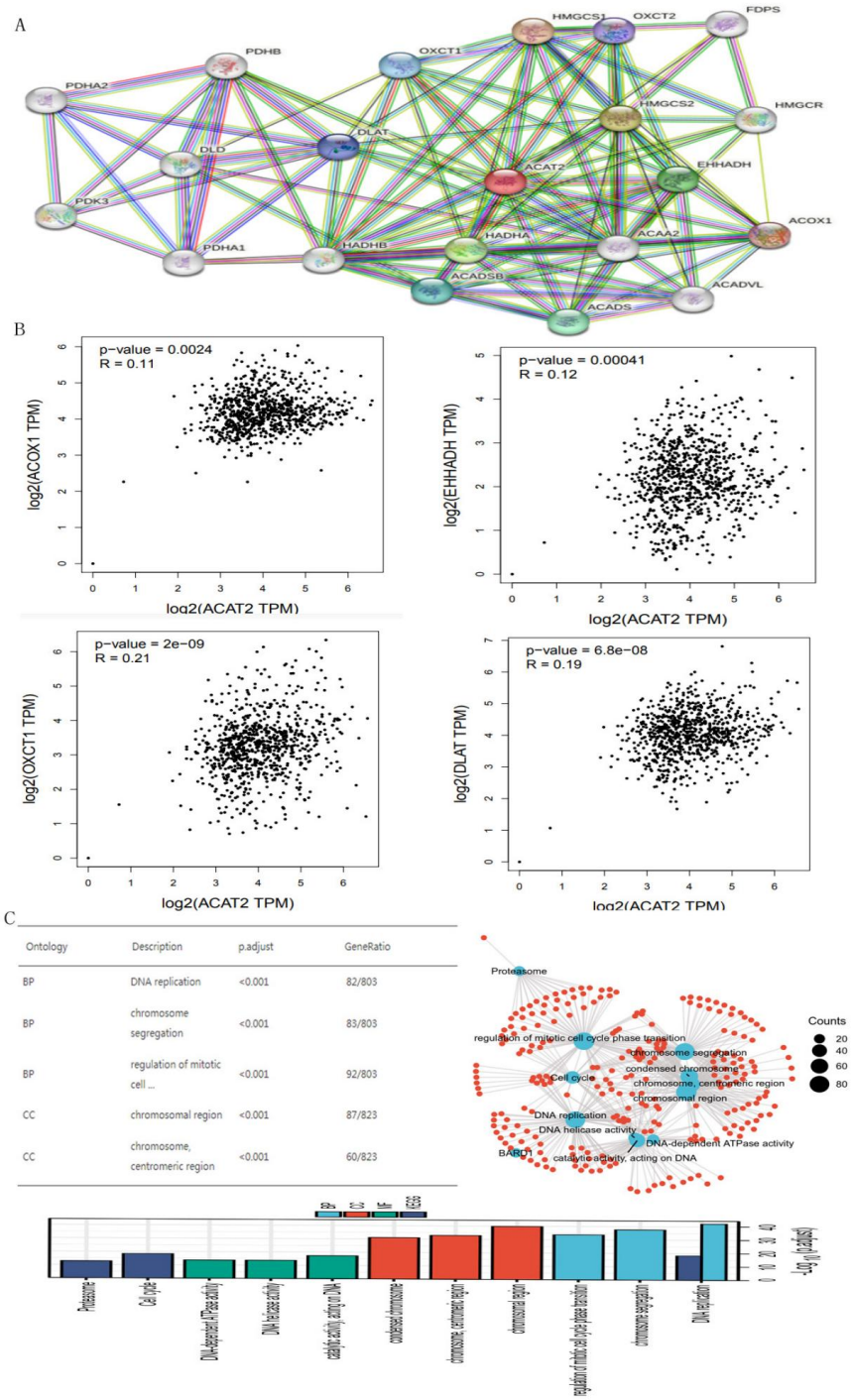

FIGURE 4 Analysis of ACAT2-correlated co-expression network and enrichment pathway

A. STRING showed ACAT2-binding molecules . B. GEPIA2 was used to analyze 4 of ACAT2-correlated genes. C. Chart of the GO and KEGG enriched pathways.

4. DISCUSSION

Acetyl-CoA acetyltransferase (ACAT) plays pivotal parts in the cholesterol metabolism pathway. ACAT family includes ACAT1 and ACAT2 two isoforms. ACAT1 participate in the metabolism of ketogenesis, and is localised in mitochondrial matrix. ACAT1 is widely expressed in multiple tumor types while ACAT2 is predominantly distributed in some organs like small intestine and liver[9]. Its mutations are reported to contribute to a wide variety of diseases[10]. Insulin drives the development of CRC indirectly through increasing in the activity of ACAT1, the results above suggest that ACAT1 could be an effective anti-cancer target for CRC[11]. Overexpression of ACAT1 is associated with neoplastic progression and poor prognosis, such as human high-grade prostate, pancreatic and breast cancers[12,13,14].

ACAT2 is involved in cytosolic acetoacetyl-CoA production, however, very little is known about other mechanisms. Recently, a new study shows that the inflammatory responses or liver stress resulted from ACAT2 overexpression, were also inhibited the metabolic pathways of glycolytic, TG synthesis, mitochondrial-related and ketone body metabolism, but upregulated genes participated in the metabolism of cholesterol, particularly the biosynthesis pathway of bile acid[15]. ACAT2 is significantly upregulated, promoted cancer cell growth and progression of CRC[8]. However, ACAT2 is downregulated in KIRC[7].

In our results, pan-cancer analysis found that ACAT2 was overexpressed in CHOL, BRCA, ESCA, BLCA, HNSC-HPVpos, HNSC-HPVneg,UCEC, KIRC, LUAD,KIRP, LUAD, THCA, LUSC. Whereas low expression of ACAT2 was observed in HNSC, LIHC, PRAD, STAD. The findings revealed that ACAT2 might play distinct parts during the course of tumorigenesis. In addition, high expression of ACAT2 had a poor DFS and OS in LUAD. Univariate and multivariate Cox regression analysis indicated that the poor outcomes might be related to the tumour stage, nodal stage, distant metastatic stage. OS of the area under the ROC curve was 0.605, 0.582, and 0.523 for 1-, 3-, and 5-year periods, respectively. This revealed that ACAT2 might be a predictive biomarker of unfavorable outcomes.

GO enrichment analysis elucidated that ACAT2 participated in biological process of cell cycle, DNA repair, DNA damage, proliferation. These processes were indispensable for maintaining diverse physiological events. If any of these processes were deregulated,cancer or other diseases might occur. Enrichment pathway analysis showed four ACAT2-correlated genes, acyl-CoA oxidase-1 (ACOX1), enoyl-CoA-hydratase/3-hydroxyacyl CoA dehydrogenase (EHHADH), 3-oxoacid CoA-transferase 1 (OXCT1), dihydrolipoamide s-acetyltransferase (DLAT). ACOX1 was a highly conserved enzyme, which played a key role in the lipid metabolism process. Inhibition of ACOX1 caused multiple metabolic disorders by improving the metabolism of reactive oxygen species (ROS) and mitochondrial lipid[16]. EHHADH participated in fatty acids degradation. A recent study found that EHHADH depletion restrained proliferation , invasion, and migration of cancer cells, facilitated the sensitivity of bladder cancer(BC) cells to cisplatin in vitro[17]. OXCT1 participated in the metabolism of ketone body and production ATP, which was an important rate-limiting enzyme[18]. Compared with the low OXCT1 expression group, the patients' relapse-free survival (RFS) was substantially shorter in high OXCT1 expression group[19]. DLAT was highly expressed in HCC specimens, high DLAT expression was correlated with poor prognosis[20].

5. Conclusion

In summary, ACAT2 is upregulated in LUAD tissues. High expression of ACAT2 has a poor clinical prognosis. We will further investigate the mechanisms of ACAT2.

Nevertheless, there are some limitations to our study. First, the molecular mechanisms of ACAT2 have not been explored. Second, it is essential to explore the potential mechanisms of ACAT2 in vivo and in vitro.

REFERENCE

1. A Amatu, A Sartore-Bianchi, K Bencardino, et al. Tropomyosin receptor kinase (TRK) biology and the role of NTRK gene fusions in cancer. Ann Oncol. 2019,30(Suppl_8):viii5-viii15.
2. Yuan Z, Jianbo C, Yunan Z, et al. Ceramide pathway regulators predict clinical prognostic risk and affect the tumor immune microenvironment in lung adenocarcinoma. Front Oncol. 2020,27,10:562574-562588.

3. Hunain A, Ming T, Mayinuer M, et al. KMT2D deficiency impairs super-enhancers to confer a glycolytic vulnerability in lung cancer. Cancer Cell. 2020,37(4):599-617.e7.
4. Bemlih S, Poirier MD, El Andaloussi A. Acyl-coenzyme A: cholesterol acyltransferase inhibitor avasimibe affect survival and proliferation of glioma tumor cell lines. Cancer Biol Ther. 2010,9(12):1025-1032.
5. Sakashita N, Miyazaki A, Takeya M, et al. Localization of human acyl-coenzyme A: cholesterol acyltransferase-1 (ACAT-1) in macrophages and in various tissues. Am J Pathol. 2000,156(1):227-236.
6. Sakashita N, Miyazaki A, Chang CC, et al. Acyl-coenzyme A: cholesterol acyltransferase 2 (ACAT2) is induced in monocyte-derived macrophages: in vivo and in vitro studies. Lab Invest. 2003,83(11):1569-1581.
7. Zhao Z, Lu J, Han L, et al. Prognostic significance of two lipid metabolism enzymes, HADHA and ACAT2, in clear cell renal cell carcinoma. Tumor Biol. 2015,37(6):8121-8130.
8. Meilin W, Hao Z, Wenting H, et al. ACAT2 promotes cell proliferation and associates with malignant progression in colorectal cancer. Onco Targets Ther. 2020,13,3477-3488.
9. Wei Y, Yibing B, Ying X, et al. Potentiating the antitumour response of CD8(+) T cells by modulating cholesterol metabolism. Nature.2016 ,531(7596):651-655.
10. Valerio D, Massimo B, Daniele S, et al. The TDH-GCN5L1-Fbxo15-KBP axis limits mitochondrial biogenesis in mouse embryonic stem cells. Nat Cell Biol. 2017,9(4):341-351.
11. Goudarzi A. The recent insights into the function of ACAT1: A possible anti-cancer therapeutic target. Life Sci. 2019;232:116592.
12. Yue S, Li J, Lee SY, et al. Cholesteryl ester accumulation induced by PTEN loss and PI3K/AKT activation underlies human prostate cancer aggressiveness. Cell Metab. 2014;19(3):393-406.
13. Li J, Gu D, Lee SS, Song B, et al. Abrogating cholesterol esterification suppresses growth and metastasis of pancreatic cancer. Oncogene. 2016;35(50):6378-6388.
14. Degonzalo-Calvo D, López-Vilaró L, Nasarre L, et al. Intratumor cholesteryl ester accumulation is associated with human breast cancer proliferation and aggressive potential: a molecular and clinicopathological study. BMC Cancer. 2015;15:460.
15. Zhimin M, Zhengyun H, Chi Z, et al. Hepatic Acat2 overexpression promotes systemic cholesterol metabolism and adipose lipid metabolism in mice. Diabetologia. 2022,15:1-16.
16. Zeng J, Deng SW, Wang YP, et al. Specific inhibition of acyl-CoA oxidase-1 by an acetylenic acid improves hepatic lipid and reactive oxygen species (ROS) metabolism in Rats fed a high fat diet. Journal of Biological Chemistry. 2017,292(9),3800-3809.
17. Shunsuke O, Hirofumi Y, Kazuki K, et al. EHHADH contributes to cisplatin resistance through regulation by tumor-suppressive microRNAs in bladder cancer. BMC Cancer. 2021,21(1):48-61.
18. Zhang S, Xie C. The role of OXCT1 in the pathogenesis of cancer as a rate-limiting enzyme of ketone body metabolism. Life Sci. 2017,183:110-115.
19. Jinsheng D, Hui L, Yang L, et al. OXCT1 enhances gemcitabine resistance through NF-κB pathway in pancreatic ductal adenocarcinoma. Front Oncol. 2021,11:698302-698315.
20. Wendong B, Junyu L, Miao L, er al. A Novel Cuproptosis-related signature identified DLAT as a prognostic biomarker for hepatocellular carcinoma patients. World J Oncol. 2022 ,13(5):299-310.