ncRNAs-mediated high express of LPCAT1 correlates with poor prognosis and expression of tumor-related signaling pathway and tumor-related gene in breast cancer

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Abstract

Breast cancer is the most common malignant tumor and ranks as the leading cause of cancer-related death among women. Although endocrine and targeted therapy have obtained positive curative effects, the high recurrence rate and mortality associated with drug resistance remain obstacles. Solid evidence indicates that lysophosphatidylcholine acyltransferase 1 (LPCAT1) plays a key role during tumorigenesis. Notably, LPCAT1 upregulates cancer-related Erbb signaling pathways by affecting the lipid microenvironment around the cell membrane. However, its function and mechanism in breast cancer are still elusive. The regulation of long noncoding RNAs (lncRNAs) on multiple molecules is closely related to the occurrence and development of breast cancer. At present, most studies contend that lncRNAs facilitate downstream target gene expression by regulating ceRNAs, while others suggest that lncRNAs may function as upstream modulators, inhibiting gene expression by promoting splicing of per-miRNAs. In this study, the expression and prognosis of LPCAT1 and noncoding RNA (LINC01176) were analyzed in multiple tumors. Data in The Genotype-Tissue Expression (GTEx) indicated that LPCAT1 may be a potential oncogene in breast cancer, while LINC01176, as a new noncoding RNA, may have an inhibitory effect on breast cancer. A series of bioinformatic analyses, including expression, correlation, and prognostic analyses, confirmed that the expression of LPCAT1 is related to the regulation of the noncoding RNA (lncRNA) LINC01176. Finally, the LINC01176/hsa-miR-218-5p/LPCAT1 axis was identified as the most likely upstream lncRNA-related pathway for LPCAT1 in breast cancer. Mechanistically, we found that LPCAT1, LINC01176, and hsa-miR-218-5p are related to various tumor-related signaling pathways by KEGG enrichment analysis, including the Erbb signaling pathway, which is closely related to breast cancer, and tumor angiogenesis-related VEGF and Notch signaling pathways. Moreover, the LPCAT1 level was significantly positively associated with HER2, PC, VEGF, and NOTCH, while LINC was significantly negatively associated with HER2, PC, and NOTCH. In summary, our study suggests that LPCAT1 contributes to poor prognosis in breast cancer and that we can improve the prognosis of breast cancer by regulating the LINC01176/hsa-miR-218-5p/LPCAT1 axis.

1. Introduction

As the most common malignant tumor, breast cancer is the leading cause of cancer-related death and remains a major health threat among women.[1] Breast cancer is a heterogeneous disease, and varied expression levels of ER, PR, and HER2 are observed even in the same patient.[2] The expression of ER is the most important factor in the majority of breast cancers.[3] The ER signaling pathway is regulated by membrane receptor tyrosine kinases, including epidermal growth factor receptor (EGFR) and HER2.[3] Breast cancer can be classified into different biological subtypes, including luminal type A, luminal type B, HER2 + type[4] and triple-negative breast cancer (TNBC), according to the four elements ER, PR, HER2 and Ki67[5, 6]. Target therapies based on different molecular pathological subtypes of breast cancer have achieved promising results in the clinic, and the prognosis of some patients has been significantly improved.[7] However, at present, there is obvious drug resistance in both endocrine therapy[8] and HER2-targeted therapy[9]. In addition, as breast tumors grow, the secretion of a considerable number of
unbalanced factors is able to trigger the formation of new vessels through the process of angiogenesis. One of the major factors is vascular endothelial growth Factor A (VEGFA) and VEGFA is also a validated target for antiangiogenic therapy in the clinic.[10] However, many studies have observed that tumor vessels can develop resistance to anti-VEGF therapy.[11] The recurrence rate and mortality rate of breast cancer are still high.[12] Therefore, there is an urgent need to develop effective therapeutic targets or promising biomarkers of breast cancer.

Lysophosphatidylcholine acyltransferases (LPCATS) function as important modulators in lipid metabolism and homeostasis.[13] Biofilms mainly consist of phospholipids. The fatty acyl chain composition of phospholipids determines the membrane biophysical characteristic, which alters its effect on biological processes.[13] The LPCAT family regulates well-known tumor targets, such as EGFR, by affecting the lipid microenvironment of the cell membrane.[14] LPCAT1, as a member of its family, is closely related to the occurrence and development of tumors[13], and it has been reported that LPCAT1 promotes the (skin squamous carcinoma)[15] malignant phenotype and may indicate poor prognosis of hepatocellular carcinoma,[16] prostate cancer,[17] and pancreatic cancer.[18] LPCAT1 upregulates the PI3K/AKT/MYC pathway, leading to brain metastasis in lung cancer.[19] More significantly, LPCAT1 can promote the localization of EGFR on the cell membrane by regulating the lipid composition of the cell membrane, leading to the development of melanoma.[20] However, in breast cancer, although it was previously believed that LPCAT1 could lead to its poor prognosis, its sample size was small, and there was no clear guiding significance. The specific mechanism by which LPCAT1 leads to poor prognosis in breast cancer remains unclear.

Noncoding RNAs are mainly composed of microRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs).[21] They are all transcribed from the genome and perform biological functions by transcription modification at the RNA level. It is worth noting that greater stability in vivo makes it a promising potential biomarker for diagnosis, prognosis and clinical therapy effects.[22, 23] The regulation of long noncoding RNAs (lncRNAs) on RNAs (miRNAs) plays an important role in the occurrence and development of tumors.[24] It has been found that the EGOT-miR-32-5p-XYLTL2 axis and SERHL-miR-1269a/miR-193b-3p-BCL2L1/SYK/ARNT/CHST3/LPCAT1 axis regulated by lncRNAs are involved in hepatocellular carcinoma caused by liver cirrhosis.[25] MiR-205 can promote the prognosis of LIHC, HNSCC, and LUSC by inhibiting LPCAT1.[26]

In this study, we first performed expression and survival analyses of LPCAT1 and noncoding RNAs in various types of human cancers. Second, the regulation of LACAT by noncoding RNAs (ncRNAs), mainly microRNAs (miRNAs), and long noncoding RNAs (lncRNAs), in breast cancer, was also discussed. Finally, we determined the relationship between the expression of lncRNAs and LPCAT, peripheral lipid metabolism, angiogenesis-related pathways, and the Erbb signaling pathway in breast cancer. In summary, our results show that the upregulation of LPCAT is related to the Erbb signaling pathway and poor prognosis of breast cancer patients. We also found that the downregulation of LPCAT mediated by ncRNAs may promote the prognosis of breast cancer patients. We named this regulatory pathway the
LINC01176/hsa-miR-218-5p/LPCAT1 axis, and we can improve the prognosis of breast cancer by regulating this axis.

2. Materials and Methods

2.1 GEPIA database analysis

GEPIA (GEPIA (Gene Expression Profiling Interactive Analysis) (cancer-pku.cn)) is a web tool for cancer and normal gene-expression profiling and interactive analyses based on TCGA and The Genotype-Tissue Expression (GTEx) data.[28] The mRNA expression data of 24 cancer types (BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LICH, LUAD, LUSC, PAAD, PARD, PCPG, READ, SARC, SKCM, THCA, THYM, STAD, UCEC) were downloaded from GEPIA database, and we use GEPLA to determine LPCAT1 and IncRNA expression in different types of human cancer. p value < 0.05 was considered as statistically significant. Besides, GEPIA was used to process survival analysis for LPCAT1 in 12 different human cancer types, including OS and RFS. We also use GEPIA to analysis the prognostic values of IncRNAs in BRCA. Log rank p value < 0.05 was considered as statistically significant.

2.2 UALCAN database analysis

UALCAN database (UALCAN (uab.edu)) uses TCGA level 3 RNA-seq and clinical data from 31 cancer types, it can perform to in-depth analyses of TCGA gene expression data.[29] We use UALCAN to analysis the expression of LPCAT1 and has-miR-218-5p in BRCA of different stages, different types.

2.3 Candidate miRNA prediction

We use the gene prediction programs, including PITA(http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html/), RNA22(RM2Target | A comprehensive database for the target genes of WERs of RNA modifications (canceromics.org)), miRNAmap(miRNAMap - Database Commons (cncb.ac.cn)), miRanda(miRanldb - Database Commons (cncb.ac.cn)) and miRTarbase(miRTarBase - Database Commons (cncb.ac.cn)) to predict the upstream miRNAs which can bind LPCAT1. The candidate miRNAs of LPCAT1 that use for subsequent analyses must appear in more than two programs.

2.4 starBase database analysis

starBase (ENCORI: The Encyclopedia of RNA Interactomes. (sysu.edu.cn)) is a database for exploring miRNA-related studies[30, 31]. We use starBase to perform the expression correlation analysis for miRNA-LPCAT1, IncRNA-has-miR-218-5p and IncRNA-LPCAT1 in BRCA. The starBase was also used to analysis the expression leve of has-miR-218-5p in BRCA and normal. In additional, we use starBase to predict the candidate IncRNAs which might bind to has-miR-218-5p.

2.5 Kaplan-Meier plotter analysis
Kaplan-Meier plotter analysis (Kaplan-Meier plotter (kmplot.com)), is an online database, it can analysis the effects of genes or miRNAs on survival in more than 20 cancer types including BRCA, we use it to conduct survival analysis for has-miR-218-5p in BRCA. Log rank p value < 0.05 was considered as statistically significant.

2.6 Gene set Enrichment analysis

Gene set enrichment (GSE) analysis is an essential tool in extracting biological insight from genome-scale experiments.[32] We use it to analysis the relationship between LPCAT1 and tumor-related signal pathway.

2.7 Quantitative real-time PCR (qRT-PCR)

The qRT-PCR was used for analyze the expression level of LPCAT1. qRT-PCR was conducted using the Go-Taq system (Promega, Madison, WI, 487 USA) under the conditions detailed in a previous study.[33]

2.8 DIANA-mirPath

DIANA-mirPath(DIANA TOOLS - mirPath (athena-innovation.gr)) is a miRNA pathway analysis web-server, providing accurate statistics, while being able to accommodate advanced pipelines. mirPath can utilize predicted miRNA targets (in CDS or 3'-UTR regions) provided by the DIANA-microT-CDS algorithm.[34] We use it to analyzed the correlation between has-miR-218-5p and tumor-related pathways, tumor-related genes in BRCA.

2.9 Statistical analysis

The statistical analysis in this study was automatically calculated by the online database mentioned above. p value < 0.05 or log rank p value < 0.05 was considered as statistically significant.

3. Results

3.1 Pancancer analysis of LPCAT expression

First, we investigated the function of LPCAT in tumors. We analyzed the expression of LPCAT1 in 24 human cancers. As shown in Fig. 1A, compared with the adjacent tissues, the expression of LPCAT1 was upregulated in 19 kinds of tumor tissues, including BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LICH, PARD, PCPG, READ, SARC, SKCM, THCA, STAD, and UCEC SKCM, while those of 5 kinds of tumors were decreased, including KICH, LUAD, LUSC, PAAD, and THYM. There was no significant difference in CESC, KICH, LUAD, LUSC, PAAD, SKCM, or THYM. Then, we further verified the expression of LPCAT1 in these 24 cancers based on the GEPIA and UALCAN databases. As presented in Fig. 1B-1X. It is suggested that LPCAT1 may play an important regulatory role in the occurrence and development of 11 cancers. Next, we analyzed the relationship between LPCAT1 and BRCA and found that LPACT1 is mainly expressed in HER2-positive and triple-negative breast cancer, while there was no correlation between LPCAT1 and lymph node metastasis or cancer stage. (Figure S1A-1D) RT-PCR indicated that the
expression of LPCAT1 in breast tumor tissue was upregulated compared with that in adjacent normal tissue. (Figure S1-E) Regardless of breast cancer cells and tissues, LPCAT1 had abnormally high expression. Therefore, we infer that it may play an important role in the occurrence and development of breast cancer.

3.2 The prognostic values of LPCAT1 in human cancer

Then, we analyzed the effect of LPCAT1 on tumor prognosis in BRCA and GBM. We analyzed overall survival (OS) and disease-free survival (RF). The results showed that the increased expression of LPCAT1 in breast cancer had a negative effect on OS, but it had no effect on RFs. Therefore, we infer that LPCAT1 may become a biomarker of poor prognosis in breast cancer patients. (Fig. 2A-L)

3.3 Prediction and analysis of upstream miRNAs of LPCAT1

It has been reported that noncoding RNAs extensively regulate a large number of genes in tumors. To determine whether LPCAT1 is regulated by noncoding RNA, we first predicted 11 upstream miRNAs that may bind to LPCAT1. Cytoscape software was used to construct a miRNA-LPCAT1 network regulation diagram (Fig. 3A). We inferred that LPCAT1 was negatively correlated with miRNA according to the regulatory mechanism of miRNA on gene expression. Then, we performed expression correlation analysis (Fig. 3B) and selected hsa-miR-218-5p, hsa-miR-128-3p, and hsa-miR-340-5p. We found that LPCAT1 was significantly negatively correlated with these three miRNAs in BRCA. Finally, we determined the expression of three miRNAs in BRCA. As shown in Fig. 3C-3E, we found that hsa-miR-128-5p has a low expression level in BRCA. Then, we further analyzed the relationship between the expression of hsa-218-5p and individual cancer stages, subclasses, and nodal metastasis status in BRCA (Fig. 3F-3H). We found that the low expression of hsa-miR-128-5p led to HER2-positive BRCA and triple-negative breast cancer (TNBC) as well as lymphatic metastasis in BRCA. All the evidence proves that hsa-218-5p might be one of the most potential regulatory miRNAs of LPCAT1 in BRCA.

The miRNA-LPCAT1 regulatory network established by Cytoscape software. (A) The expression correlation between predicted miRNAs and LPCAT1 in BRCA analyzed by the starBase database. (B) The expression of hsa-miR-128-3p in BRCA and normal samples determined by the starBase database. (C) The expression of hsa-miR-128-5p in BRCA and normal samples determined by the starBase database. (D) The expression of hsa-miR-340-3p in BRCA and normal samples determined by the starBase database. (E) The prognostic value of hsa-miR-128-5p in BRCA (F-H) *P value < 0.05, **P < 0.01, ***P < 0.001.

3.4 Prediction and analysis of IncRNAs upstream of hsa-miR-218-5p

Then, we predicted the upstream IncRNAs of hsa-218-miR-5p by using the starBase database and determined the expression levels of upstream IncRNAs in BRCA. We discovered that MIR99AHG, LINC01176, NEAT1, and SCAMP1-AS1 were significantly downregulated in BRCA compared with normal tissues from all 36 predicted IncRNAs (Fig. 4A-4D). After that, the prognostic values of these IncRNAs in
BRCA were analyzed. (Fig. 4E-4L) We found that BRCA patients with higher expression of LINC01176 had improved OS. In addition, ERB-b2 is a key breast cancer target,[35] and DGCR8 is an important regulatory gene of lipid metabolism.[36] The ERBB2 receptor in the ERBB signaling pathway is regulated by lipid metabolism as a cell membrane receptor.[37] DGCR8 can indirectly regulate ERBB. It has been reported that high expression of Her2 causes poor prognosis of cancer[38] while high expression of DGCR8 causes the opposite result[38]. After verification of the relationship between LINC01176 and DGCR8 and HEB-b2, we found that LINC01176 has a positive correlation with DGCR8 but a negative correlation with HEB-b2 (Fig. 5J, 5K). Combined with OS analysis, we concluded that LINC01176 can improve the prognosis of patients with BRCA. Next, by analyzing the data, we found a negative correlation between IncRNAs and RNAs and a positive correlation between IncRNAs and miRNAs. (Fig. 5A-5I) Different from the ceRNA hypothesis, some studies have found that IncRNAs can promote the splicing of pri-miRNA and sometimes even become precursors of miRNA, finally inhibiting the target mRNA.[39] We can infer that LINC01176 can reduce the expression of LPCAT1 by increasing the expression of hsa-miR-218-5p, ultimately improving the prognosis of patients with BRCA.

The expression of MIR99AHG (A), LINC01176 (B), NEAT1 (C), and SCAMP-AS1 (D) in BRCA compared with normal tissues. OS analysis for MIR99AG (E), LINC01176 (F), NEAT1 (G), and SCAMP-AS1 (H) in BRCA. The RFS for MIR99AG (I), LINC01176 (J), NEAT1 (K), and SCAMP-AS1 (L) in BRCA.

Correlation analysis between LPCAT1 and MIR99AHG (A), LINC01176 (B), NEAT1 (D), and SCAMP1-AS1 (E). Correlation analysis between hsa-miR-218-5p and MIR99AHG (F), LINC01176 (G), NEAT1 (H), and SCAMP1-AS1 (I). Correlation analysis between LINC01176 and ERB-b2 and Cgcr8. (J, K) *P value < 0.05, **P < 0.01, ***P < 0.001.

3.5 LPCAT1, hsa-miR-218-5p and LINC01176 are correlated with the tumor-related signaling pathway and tumor-related genes

Many signaling pathways are related to tumors, such as the ERB-b signaling pathway[40]; the metabolic signaling pathways related to tumors, including glycosphingolipid biosynthesis lacto and neolacto series[41], the phosphatidylinositol signaling system[42], and inositol phosphate metabolism[43]; and the key signaling pathways for tumor angiogenesis, including the NOTCH signaling pathway[44], and the VEGF signaling pathway[10]. By using GSEA, we found that high expression of LPCAT1 increased the correlation with these tumor-related pathways (Fig. 6A-6D, Fig. 7A, 7B). Moreover, a large number of studies have shown that many genes can affect the prognosis of BRCA, and some of them will lead to poor prognosis, such as NOTCH1[45], ERB-b2[46], NOTCH2[47], VEGFA[48], NOTCH3[49], JAG1[50], and JAG2[51]. Some will improve the prognosis; for instance, CAV1[52] CAV1 is an important protein for lipid regulation of HER2, and it can downregulate HER2. Through the data analysis, we concluded that LPCAT1 was positively correlated with ERB-b2 (P = 8e-04), VEGFA (P = 0.00078), NOTCH1 (P = 0.018), JAG2 (P = 0.031), and NOTCH2 (0.011), while LPCAT1 was negatively correlated with CAV1 (P = 0.035). The relationship between LPCAT1 and NOTCH3, JAG1, and NOTCH4 was not statistically significant.
Finally, we verified the correlation between hsa-miR-218-5p and LINC0176 with these signaling pathways and the genes above, and we obtained the opposite result. These results demonstrate that LPCAT1 might lead to poor prognosis in patients with BRCA by regulating the tumor-related signaling pathway and tumor-related genes. The expression of hsa-miR-218-5p and LINC01176 can change this adverse effect.

Correlation analysis between LPCAT1 and glycosphingolipid biosynthesis lacto and neolacto series (6A), phosphatidylinositol signal system (6B), inositol phosphate metabolism (6C), ERB-b signaling pathway (6D), NOTCH signaling pathway (7A), and VEGF signaling pathway (7B). *P value < 0.05, **P < 0.01, ***P < 0.001.

Figure 6, 7: Correlation analysis between LPCAT1 and tumor-related genes in BRCA

Correlation analysis between LPCAT1 and ERB-b2 (6E), CAV1 (6F), VGEFA (7C), NOTCH1 (7D), NOTCH2 (7E), JAG1 (7F), JAG2 (7G), NOTCH4 (7H), and NOTCH3 (7I) in patients with BRCA. *P value < 0.05, **P < 0.01, ***P < 0.001.

4. Discussion

Today, BRCA still plagues many women. Explaining the molecular mechanism of BRCA carcinogenesis can provide important clues for finding effective therapeutic targets and seeking prognostic biomarkers. Increasing evidence has proven that LPCAT1 plays an important role in the occurrence and development of many human cancers, including BRCA. The study of LPCAT1 in BRCA is still lacking and needs to be further studied.

In this investigation, we first used The GEPIA database to perform pancancer analysis of LPCAT1 expression. Next, we validated LPCAT1 expression by using the GEPIA database. Through survival analysis for LPCAT1 in many selected cancer types, we found that BRCA patients with high expression of LPCAT1 had poorer prognosis than patients with low expression of LPCAT1. Our study results indicated that LPCAT1 has a carcinogenic effect in BRCA.

Many studies prove that ncRNAs, including IncRNAs, miRNAs, and circular RNAs (circRNAs), can participate in the regulation of gene expression through the ceRNA mechanism[53][54]. However, in this study, we found that IncRNAs can promote the expression of miRNAs and inhibit target gene mRNAs. First, to identify the upstream regulatory miRNAs of LPCAT1, we utilized prediction programs to predict the possible miRNAs that can bind to LPCAT1. Through the programs, we finally obtained 12 miRNAs. Some of these have been proven to play a key role in BRCA, such as hsa-miR-205[54]. Through expression analysis, correlation analysis and survival analysis, we finally determined that hsa-miR-218-5p is the most likely upstream tumor suppressive miRNA of LPCAT1.

The IncRNAs of the hsa-miR-218-5p/LPCAT1 axis might be tumor suppressor IncRNAs in BRCA. Then, we predicted upstream IncRNAs of the hsa-miR-218-5p/LPCAT1 axis and found 36 IncRNAs. We finally
determined the four most potentially upregulated IncRNAs, including MIR99AHG, LINC01176, NEAT1, and SCAMP1-AS1, by expression analysis, correlation analysis, and survival analysis. These four IncRNAs have been found to play an important role in many cancers. For example, miR99AHG is a noncoding tumor suppressor gene in lung adenocarcinoma[55] but it can also accelerate pancreatic cancer[56]. NEAT1 can promote bone metastasis of prostate cancer[57] and NEAT1 can also promote colorectal cancer. SCAMP1-AS1 can become a biomarker of N2 metastasis in T1 lung adenocarcinoma[58]. We found that only LINC01176 can improve the prognosis of patients with BRCA. In summary, the LINC01176/hsa-miR-218-5p/LPCAT1 axis might be identified as one of the regulatory pathways in BRCA.

LPACT1 mainly affects lipid metabolism[20]; EHR2, as a membrane receptor in the ERBB signaling pathway, receives lipid regulation, so LPACT1 can indirectly affect HER2. Our work suggested that LPCAT1 was significantly positively correlated with the ERB-b signaling pathway and lipid metabolism signaling pathway, including the glycosphingolipid biosynthesis lacto and neolacto series, phosphatidylinositol signaling system, and inositol phosphate metabolism. Angiogenesis is an important factor affecting breast tumor transmission and development, and VEGF and NOTCH are the most important factors for tumor angiogenesis[59]. We also found that LPCAT1 was significantly positively correlated with the NOTCH signaling pathway and VEGF signaling pathway. In addition, LPCAT1 was positively correlated with ERB-b2, VEGFA, NOTCH1, JAG2, and NOTCH2 but negatively correlated with CAV1.

In summary, we explained that LPCAT1 had a high expression level in many types of human cancer, including BRCA, and we also found that it positively correlated with poor prognosis in BRCA. We identified an upstream regulatory mechanism of LPCAT1 in BRCA, which we call the LINC01176/has-miR-218-5p/LPCAT1 axis, and we can improve the prognosis of breast cancer by regulating this axis.

Declarations

Statements and Declarations

Acknowledgement

Not applicable

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Competing interests

The authors have no relevant financial or non-financial interests to disclose

Author Contributions
Junhao Mu and Rong Ma designed this work. Yuezhou Zhang performed bioinformatic analyses and wrote the manuscript. Yu Fan, Siyuan Zheng and Minjie Zhao, made the figure in the manuscript. Jiefu Luo and Junyan Liu revised the manuscript.

**Data Availability**

The datasets used and analyzed during the study are available from the corresponding authors on reasonable request. The database including: GEPIA (GEPIA (Gene Expression Profiling Interactive Analysis) (cancer-pku.cn)), UALCAN database (UALCAN (uab.edu)), PITA(http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html/1), RNA22(RM2Target | A comprehensive database for the target genes of WERs of RNA modifications (canceromics.org)), miRNAmap(miRNAmap - Database Commons (cnbc.ac.cn)), miRanda(miRandb - Database Commons (cnbc.ac.cn)) miRTarbase(miRTarBase - Database Commons (cnbc.ac.cn)), starBase (ENCORI: The Encyclopedia of RNA Interactomes. (sysu.edu.cn)), Kaplan-Meier plotter analysis (Kaplan-Meier plotter (kmplot.com)), DIANA-mirPath(DIANA TOOLS - mirPath (athena-innovation.gr))

**Ethics approval**

This is an observational study. The Research Ethics Committee has confirmed that no ethical approval is required. We didn’t use any human participant in our study.

**Consent to publish**

Not applicable

**References**


Figures
Figure 1

Expression analysis for LPCAT1 in multiple cancers

(A) The expression of LPCAT1 in 24 types of human cancer based on GEPIA cancer and normal data. LPCAT1 expression in GEPIA BLCA (B), BRCA (C), CESC (D), CHOL (E), COAD (F), ESCA (G), GBM (H), HNSC (I), KICH (J), KIRC (K), PRAD (L), LICH (M), LUAD (N), LUSC (O), PAAD (P), PARD (Q), PCPG (R),
READ (S), SARC (T), SKCM (U), THYM (V), STAD (W) AND UCEC (X) tissues compared with corresponding GTEx normal tissues. *P value < 0.05; **P value < 0.01; ***P value < 0.001.

Figure 2

The overall survival (OS) analysis for LPCAT1 in various human cancers determined by the GEPIA database
The OS plot of BLCA(A), BRCA(B), CHOL(C), GBM(D), KICH(E), KIRC(F), ESCA(G), READ(H), HNSC(I), PAAD(J), SKCM(K), and STAD(L).

![Graph showing OS plot for various types of cancer]

Figure 3

Identification of hsa-miR-218-5p as a potential upstream miRNA of LPCAT1 in BRCA.

The miRNA-LPCAT1 regulatory network established by Cytoscape software. (A) The expression correlation between predicted miRNAs and LPCAT1 in BRCA analyzed by the starBase database. (B) The expression of hsa-miR-128-3p in BRCA and normal samples determined by the starBase database. (C) The expression of hsa-miR-128-5p in BRCA and normal samples determined by the starBase database. (D) The expression of hsa-miR-340-3p in BRCA and normal samples determined by the starBase database.

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Figure 4

Expression analysis and survival analysis for upstream lncRNAs of hsa-miR-218-5p in BRCA
Figure 5

Correlation analysis between lncRNAs and hsa-miR-218-5p or IncRNAs and LPCAT1 in BRCA determined by starBase and GEPIA database. (C) Correlation analysis between LINC01176 and two prognostic influencing factors in BRCA.

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<td>LINC01176</td>
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<td>0.110*</td>
<td>2.81e-04***</td>
</tr>
<tr>
<td>NEAT1</td>
<td>hsa-miR-218-5p</td>
<td>-0.01</td>
<td>7.47e-01***</td>
</tr>
<tr>
<td>SCAMP1-AS1</td>
<td>hsa-miR-218-5p</td>
<td>0.070</td>
<td>2.06e-02***</td>
</tr>
</tbody>
</table>

*These results are statistically significant.

* p value < 0.05, ** p value < 0.01, *** p value < 0.001
SCAMP1-AS1 (I). Correlation analysis between LINC01176 and ERB-b2 and Cgcr8. (J, K) *P value < 0.05, **P<0.01, ***P<0.001.

Figure 6
Correlation analysis between LPCAT1 and tumor-related pathways in BRCA by gene set enrichment analysis
Figure 7

Correlation analysis between LPCAT1 and tumor-related genes in BRCA

Correlation analysis between LPCAT1 and ERB-b2 (6E), CAV1 (6F), VGEFA (7C), NOTCH1 (7D), NOTCH2 (7E), JAG1 (7F), JAG2 (7G), NOTCH4 (7H), and NOTCH3 (7I) in patients with BRCA. *P value < 0.05, **P<0.01, ***P<0.001.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- sFig1.tif
- sFig2.tif
- sFig3.png
- sFig4.png
• thelegendforsfigure.docx