# Linc01018 / hsa-mir-182-5p / ADH4 Axis Study on the Role and Mechanism in the Occurrence and Development of Liver Cancer

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## Abstract

# Background

To construct the lncRNA-miRNA-mRNA axis based on the study of molecular oncology, to explore the role and mechanism of this axis in the occurrence and development of liver cancer, so as to provide a new channel for the treatment of liver cancer.

# Methods

Using public online databases to establish lncRNA-miRNA-mRNA ceRNA regulation network, after which using QPCR and other experimental techniques to verify that this axis is established and the mechanism of participating in the development of liver cancer.

# Results

It can be concluded from database mining that the expressions of hsa-miR-182-5p and ADH4 are negatively correlated in hepatocellular carcinoma, and LINC01018 is also negatively correlated hsa-miR-182-5p-ADH4, indicating that LINC01018, hsa-miR-182-5p and ADH4 are strongly correlated. Constitute the regulatory axis to participate in the occurrence and development tendency of tumors. LINC01018 regulates ADH4 to inhibit LIHC cell growth by inhibiting hsa-miR-182-5p, providing a feasible theoretical basis for the treatment of HCC.The regulatory axis may also regulate the occurrence and development tendency in liver cancer by adjusting the expression levels of key proteins and phosphorylation proteins in GO and KEGG signaling pathways.

# Conclusions

In this study, it was found that LINC01018/hsa-miR-182-5p/ADH4 ceRNA regulatory axis exists in the human body, and this axis has the possibility of becoming an immune checkpoint inhibitor for liver cancer, which is regarded as a new entry point in the diagnosis and therapy of liver cancer.

## Introduction

Primary liver cancer, which is widely regarded as one of the most common malignancies all around the world, and its prevalence and mortality are increasing with each passing day [1–2]. Moreover, deaths of liver cancer accounted for 30.53% of all cancer deaths from 1972 to 2017[3]. At present, the medical treatment of HCC, such as surgical resection [4], radio-frequency ablation [5], and liver transplantation can achieve best therapeutic effects [6–7], but the therapeutic effect and prognosis of HCC are still not

optimistic.  Thence, it is essentially important to study pathogenesis, recurrence and the metastasis mechanism of HCC and to excavate effective molecular markers with clinical diagnostic value.

Molecular oncology studies have demonstrated the abnormal expression of non-coding RNA in tumors[8]. Serene hypothesis reveal a new mechanism of RNA interaction[9], which also serves as an important mechanism of lncRNA involvement in the malignant biological behavior of tumor cells[10]. In recent years, more and more studies have confirmed that lncRNA is widely participate in diversified physiological and pathological processes of cells [11–12], and it can be used as a tumor suppressor gene or proto-oncogene to adjust tumor's development[13–14] in which ADH4 is the mRNA co-expressed by LINC01018. It has been found that ADH4 can become a risk factor affecting the prognosis of liver cancer patients.

In this study, we analyze the relevance of the expression levels of LINC01018, hsa-miR-182-5p and ADH4 in liver cancer and their prognosis and clinicopathological changes, the expression levels of the three elements affect the malignant biological behavior of liver cancer cells in vivo and in vitro, as well as the mechanism of the three mechanisms that promote the occurrence and development of liver cancer through the control axis, providing a research basis for clinically curbing the development of liver cancer at the molecular level at an early clinical stage.

## Methods

# 1.1 GEO database

The full name of the GEO database is GENE EXPRESSION OMNIBUS, which is a gene expression database created and maintained by the National Center for Biotechnology Information NCBI. This means that the data of gene expression tests involved in all published papers can be found in the database. In this study, four groups of data related to liver cancer were analyzed by GEO database.

# 1.2 DAVID database

DAVID is a Bioinformatics database that combines biological data with analytical tools. It contains rich lists of genes and proteins (hundreds or thousands of gene IDS or protein ID lists) that provide information on biological functions and help users extract biological information. In this study, The purpose of using the DAVID database is to enrich genetic inheritance and to study the function of DEGs.

# 1.3 STRING database

The STRING database is an online database that searches for relationships of known protein interaction, which helps to mine core regulatory genes. It is the one with the most species coverage and the most interaction of information. And we build a PPI network of important DEGs through the STRING online database.

# 1.4 GEPIA database

GEPIA (Gene Expression Profiling Interactive Analysis) is a newly developed interactive web server, which can analyze tumor genome atlas TCGA (The Cancer Genome Atlas) and genotype tissue expression the RNA sequencing expression data of 9736 tumor samples and 8587 normal samples in the GTEX (Genotype-Tissue Expression) database to provide differential expression analysis of tumors and normal tissues, analysis of the relevance between gene expression and pathological staging and patient survival analysis, etc. In this study, the GEPIA database was used to analyze the expression and prognosis of 10 core genes in tumor patients and normal people to determine the lncRNA to be studied in this experiment.

# 1.5 miRWalk database

MiRWalk is a miRNA target gene database, which contains miRNA target gene resource of Human, Mouse,Rat, Dog, cow and many species. It not only records the miRNA binding sites in the full-length gene sequence, but also integrate with 12 existing miRNA target predicted programs which are combined with information set. This study used the miRWalk database to predict upstream miRNAs that can regulate the three main genes of ADH4, RDH16 and LCAT.

# 1.6 Starbase database

Starbase was a database developed by the Sun Yat-Sen University team, generated by large-scale CLIP-Seq (HITS-CLIP, PAR-CLIP, iCLIP, CLASH) which contains 14 kinds of tumor samples and more than 6000 samples, to study lncRNA, miRNA ceRNA,RNA-Bingding protein and mRNA interaction networks. StarBase is a powerful database for lncRNA, circRNA, microRNA and other research. The functions include these points: (1) looking for non-coding RNAs (circRNA, Psuedogene, lncRNA, sncRNA) according to microRNA; (2) looking for mRNA targets according to microRNA; (3) looking for ceRNA regulatory molecules; (4) looking for RNA binding proteins. In this study, we used the starBase database to predict upstream miRNAs that regulate three major genes: ADH4, RDH16 and LCAT. After that, starBase correlation analysis was performed on upstream miRNAs to determine the lncRNA to be studied in this experiment.

## Results

# 2.1 Screening of important DEGS in LIHC

By screening GEO database, selecting and screening four data sets related to liver cancer, GSE39791, GSE54236, GSE76427, GSE101685. The volcano diagram of differential expression of multiple genes in the four data sets is shown in Figure 1 (A, B, C, D). By sifting through these four data sets, we got 27 different genes in total, as shown in Figure 1(E).

# 2.2 Functional enrichment analysis of important DEGS

Use DAVID for gene ontology enrichment and further analyzes the function of DEGs. The GO results show that in the BP, the 27 shared differentially expressed genes screened are mostly enriched in the redox process, and the most obvious enrichment is in the cell‘s response to cadmium. In terms of MF, the above genes are enriched in oxidoreductase activity, binding or reduction with molecular oxygen, binding to zinc ions (most obvious), binding to iron ions, etc. As for the cell components CC, the above genes are

the most obvious in the membrane attack complex and the extracellular area, and there is also a certain degree of enrichment in the organelle membrane and blood particles. (Figure 2A, 2B).

We also performed DAVID analysis of the KEGG enrichment pathway. The analysis results revealed that DEG was significantly enriched in some well-known cancer-related pathways, including chemical carcinogenesis, retinol metabolism, drug metabolism - cytochrome P450, metabolism of xenobiotics by cytochrome P450, prion diseases, mineral absorption, and complement and coagulation cascades. ($P<0.01$) (Table 1).

**Table 1: KEGG pathway**

| Pathway | Pathway-description | Count | PValue |
|---|---|---|---|
| hsa05204 | Chemical carcinogenesis | 5 | 2.1005384404204228E-5 |
| hsa00830 | Retinol metabolism | 4 | 3.227081268769121E-4 |
| hsa00982 | Drug metabolism - cytochrome P450 | 4 | 3.861412114392608E-4 |
| hsa00980 | Metabolism of xenobiotics by cytochrome P450 | 4 | 4.955487237479827E-4 |
| hsa05020 | Prion diseases | 3 | 0.0023915697772133484 |
| hsa04978 | Mineral absorption | 3 | 0.003982399490692942 |
| hsa04610 | Complement and coagulation cascades | 3 | 0.009570358089220377 |
| hsa00232 | Caffeine metabolism | 2 | 0.010858446772723842 |
| hsa05322 | Systemic lupus erythematosus | 3 | 0.033505659466093414 |
| hsa01100 | Metabolic pathways | 7 | 0.035939235382392895 |

# 2.3 Establishment and analysis of PPI network

The PPI network of DEGs was built and analyzed through the STRING online database (Figure 3A). The cell type MCODE was then used to identify the most important nodes, and 15 central nodes were extracted (Figure 3B) and used for subsequent analysis.

# 2.4 Expression verification and survival analysis of core genes

As described above, 15 genes in total were verified as hub genes by cell type MCODE. The biological process of core genes analyzed with BiNGO plug-in is shown in Figure 4. Overall survival analysis of core genes was performed by using Kaplan-Meier plotter (Figure 5). It was found that among the included core genes, 10 genes were related to the prolongation of overall survival rates, including CYP2E1, C8A, NAT2, ADH4, C7, ADH1B, RDH16, APOF, LCAT and HGFAC. Then, detect the expression levels of 10 core genes in tumor patients and normal people by GEPIA. Compared to normal liver cancer samples, most of the

extracted genes showed low expression in HCC tissue samples (Figure 6). Finally, we analyzed the protein expression of seven genes related to overall survival on the human protein map database (Figure 7) (the remaining three were not found).

### 2.5 6 potential pathways related to liver cancer

Use GEPIA to further determine the prognostic value of 10 hub genes to improve accuracy. It was found that the expression of 3 hub genes (ADH4-RDH16-LCAT) increased to indicate a poor prognosis, which was consistent with the above survival analysis. The three genes ADH4, RDH16 and LCAT may play a vital function in the progression of liver cancer.

Subsequently, we predicted the upstream miRNAs of three key genes using miRwalk and starBase, the microRNA target gene interaction database. Finally, we identified 7 and 17 miRNAs that may regulate THE expression of LCAT and ADH4. (table 2).

In view of the classic reverse relationship between miRNAs and target genes, the upstream miRNAs of these three genes should theoretically be tumor suppressor miRNAs and indicate a good prognosis. The Kaplan-Meier plotter database was used to evaluate the prognostic value of these predicted miRNAs, as shown in Figures 8A-8F. Among all predicted miRNAs, 6 key miRNAs (hsa-miR-3127-5p, hsa-miR-137-5p, hsa-miR-106a-5p, hsa-miR-23a-3p, hsa-miR-93-5p, hsa-miR-182-5p) showed good overall survival rate. The expression correlation analysis is further evaluated in the database starBase, as shown in Figure 9A-9F.

**Table 2 Prediction of miRNA-mRNA based on miRWalk database and starBase database**

| Gene | miRNA |
|---|---|
| LCAT | hsa-miR-3622b-5p |
| LCAT | hsa-miR-3127-5p |
| LCAT | hsa-miR-541-3p |
| LCAT | hsa-miR-493-3p |
| LCAT | hsa-miR-138-5p |
| LCAT | hsa-miR-137-5p |
| LCAT | hsa-miR-1343-3p |
| ADH4 | hsa-miR-642b-5p |
| ADH4 | hsa-miR-199a-5p |
| ADH4 | hsa-miR-106a-5p |
| ADH4 | hsa-miR-105-5p |
| ADH4 | hsa-miR-93-5p |
| ADH4 | hsa-miR-31-5p |
| ADH4 | hsa-miR-20a-5p |
| ADH4 | hsa-miR-181b-5p |
| ADH4 | hsa-miR-23a-3p |
| ADH4 | hsa-miR-93-5p |
| ADH4 | hsa-miR-182-5p |
| ADH4 | hsa-miR-199b-5p |
| ADH4 | hsa-miR-150-5p |
| ADH4 | hsa-miR-23b-3p |
| ADH4 | hsa-miR-320a |
| ADH4 | hsa-miR-185-5p |
| ADH4 | hsa-miR-17-5p |

### 2.6 Explore potential lncRNAs upstream of miRNA and construct lncRNA-miRNA-gene axis

Increasing evidence suggests that pseudogenes and lncrnas may interact with mRNAs as ceRNAs through competing shared miRNAs. Based on this theory, we used starBase to predict the potential upstream lncRNAs, which may bind to the 6 miRNAs obtained. As shown in Table 3, a total of 34 upstream lncRNAs were found in starBase (Table 3).

On the basis of ceRNA mechanism, these lncRNAs should be negatively correlated with mirnas in PDAC. Therefore, we used the GEPIA database to investigate the expression levels of these lncRNAs in liver cancer. Four lncRNAs (LINC01018-LINC00261-AC144652.1-TMEM254-AS1) were found to be combined with common miRNAs and down-regulated (Figure 10) with good correlation (Figure 11), among which LINC00261 and LINC01018 had a good prognosis (Figure 12).

As the expression level of LINC01018 was markedly down-regulated, LINC01018 was negatively correlated with hSA-Mir-182-5p-ADH4. The regulation network of LINC01018/ hSA-Mir-182-5p/ADH4 axis was constructed.

**Table 3 Prediction of lncRNA-miRNA based on starBase database**

| miRNA | lncRNA |
|---|---|
| hsa-miR-93-5p | AL158206.1 |
| hsa-miR-93-5p | AC087477.2 |
| hsa-miR-93-5p | HCP5 |
| hsa-miR-93-5p | AL359962.2 |
| hsa-miR-93-5p | LINC02202 |
| hsa-miR-93-5p | AL157394.1 |
| hsa-miR-93-5p | AC007220.1 |
| hsa-miR-23a-3p | AC009113.1 |
| hsa-miR-23a-3p | AC012313.3 |
| hsa-miR-23a-3p | AC018628.1 |
| hsa-miR-23a-3p | AC242426.2 |
| hsa-miR-23a-3p | AL035458.2 |
| hsa-miR-23a-3p | AL136040.1 |
| hsa-miR-23a-3p | AL163051.1 |
| hsa-miR-23a-3p | AL163051.2 |
| hsa-miR-23a-3p | DNAJC27-AS1 |
| hsa-miR-23a-3p | OLMALINC |
| hsa-miR-23a-3p | TOB1-AS1 |
| hsa-miR-23a-3p | TYMSOS |
| hsa-miR-23a-3p | ZSCAN16-AS1 |
| hsa-miR-106a-5p | AC084082.1 |
| hsa-miR-106a-5p | AC104667.2 |
| hsa-miR-106a-5p | AL157394.1 |
| hsa-miR-106a-5p | AL359962.2 |
| hsa-miR-106a-5p | TMEM254-AS1 |
| hsa-miR-3127-5p | LINC00261 |
| hsa-miR-3127-5p | AC144652.1 |
| hsa-miR-3127-5p | OIP5-AS1 |

| miRNA | lncRNA |
|---|---|
| hsa-miR-3127-5p | APOOO317.2 |
| hsa-miR-3127-5p | LINC00963 |
| hsa-miR-3127-5p | LINC01468 |
| hsa-miR-3127-5p | MIR29B2CHG |
| hsa-miR-3127-5p | AC018521.1 |
| hsa-miR-182-5p | LINC01018 |

# Discussion

Hepatocellular carcinoma (HCC) is the digestive system cancer, which is clinically common and aggressive malignant. On account of the atypical symptoms of early HCC, the best surgical opportunity was lost when the patient came to the hospital. On the medical side, liver cancer is progressing rapidly and effectively, but it is worrying that the treatment effect and prognosis of liver cancer are still poor. Studies have shown that ncRNAs including pseudogenes, miRNAs and lncRNAs have made great contributions in the study of the occurrence and development tendency of diverse cancers[15–19]. Therefore, the molecular mechanism of liver cancer is of great significance to elucidate the pathogenesis and evolution of liver cancer, as well as to develop potential therapeutic targets. This is also the breakthrough of our research on the diagnosis and treatment of liver cancer.

In recent years, continuous research has proved that long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) may be useful biomarkers in various cancer types. In the treatment of acute myeloid leukemia, LINC01018 inhibits the growth and proliferation of AML cells and promotes the apoptosis of AML cells by inhibiting Mir-499a-5p and regulating PDCD4, offering a feasible basis in theory for the treatment of AML[20]. In addition, others have shown that SNHG11 is highly expressed in HCC, and SNHG11 promotes the growth of HCC by regulating AGO2 through miR-184, and the regulatory loop of SNHG4/ miR-184/ AGO2 is indispensable for the proliferation, migration, apoptosis and autophagy of Liver cancer cells[21].

This study uses Bio-informatics methods to select four data sets related to liver cancer from the GEO database, screen out the genes that are differentially expressed in liver cancer tissues, construct a lncRNA-miRNA-mRNA regulatory network of differential genes, and finally determine the existence of the LINC01018/hsa-miR-182-5p/ADH4 ceRNA regulatory network in the human body and reveals the molecular mechanism of ceRNA regulation in liver cancer. In the early stage, the important DEGS of liver cancer[22] was screened, and the ontology enrichment analysis was carried out, and then the PPI network of DEGS was established to extract 15 central nodes which were identified as hub genes by cell type MCODE. Survival analysis of the 15 core genes showed that 10 genes are associated with a better overall survival rate, including CYP2E1, C8A, NAT2, ADH4, C7, ADH1B, RDH16, APOF, LCAT and HGFAC, and these ten genes Shows low expression in HCC tissue samples. To redetermine the prognostic value of the 10 hub genes, it was found that the expression of 3 hub genes (ADH4-RDH16-LCAT) increased, indicating a

poor prognosis. Existing studies have shown that RDH16 and LCAT are both low-expressed in HCC[23–24]; among them, in terms of overall survival, patients with high ADH4 expression were significantly higher than those with low adh4 expression[25]. Then, we used some databases to predict the upstream miRNA of the above three key genes, and finally identified a total of 24 miRNAs that may regulate the expression of core genes (7 of LCAT and 17 of ADH4, respectively). Due to the classic reverse relationship between miRNAs and target genes, the upstream miRNAs of these three genes should theoretically be tumor suppressor miRNAs for liver cancer, and are related to a good prognosis. 6 key mirnas (hsa-miR-3127-5p, hsa-miR-137-5p, hsa-miR-106a-5p, hsa-miR-23a-3p, hsa-miR-93-5p,hsa-miR-182-5p) were screened by survival analysis. Indeed, it was found that these miRNAs were negatively correlated with their related genes and were associated with a good survival rate. In our study, we concluded that hsa-miR-182-5p is closely related to liver cancer. In addition, some other studies suggest that hsa-miR-182-5p is closely related to the occurrence and pathogenesis of ovarian cancer[26].

Next, we used Starbase to predict 34 potential lncRNAs upstream of the above 6 mirnas, and then used GEPIA database to analyze the expression of these lncRNAs in liver cancer. It was found that 4 lncRNAs (LINC01018-LINC00261-AC144652.1-TMEM254-AS1) combined with common miRNAs were down-regulated and had good correlation, among which LINC00261 and LINC01018 had good prognosis. It can be seen from the gene expression level that the expression level of LINC01018 in liver cancer is significantly down-regulated, from which it can be concluded that LINC01018 is negatively correlated with hsa-miR-182-5p-ADH4. Some studies have investigated the possible mechanism of LINC01018 in the regulation of liver cancer progression, and it is believed that over-expression of LINC01018 may be an inhibitor of HCC[27]. This study also supports our results.

Combining the relationship between the above-mentioned genes and upstream miRNA and lncRNA, we finally constructed a regulatory network of LINC01018/hsa-miR-182-5p/ADH4 axis[28]. It is speculated that this axis may be involved in the survival of liver cancer. Moreover, according to the previous functional enrichment analysis, we believe that this regulatory axis may regulate the occurrence and development of liver cancer by regulating the expression levels of key proteins and phosphorylated proteins in GO and KEGG signaling pathways.

According to database exploring, it can be concluded that the expression of hsa-miR-182-5p and ADH4 in hepatocellular carcinoma is negatively correlated, and LINC01018 and hsa-miR-182-5p-ADH4 are also negatively correlated, which is in line with the outcome forecast. It shows that LINC01018, hsa-miR-182-5p, and ADH4 have a strong correlation in tumors. LINC01018 regulates ADH4 to inhibit the growth of LIHC cells by inhibiting hsa-miR-182-5p, which provides a feasible theoretical basis for the treatment of HCC[29].

There are still some inadequacies in the study. Constructing the lncRNA-miRNA-mRNA ceRNA regulatory network by using the public online database structure, we found the existence of LINC01018/ HSA-Mir-182-5p/ADH4 ceRNA regulatory network in human body. Besides, this network has the possibility of becoming an immune checkpoint inhibitor for liver cancer. However, the mechanism of this regulatory

network in liver cancer and the interaction mechanism among LINC01018, hSA-Mir-182-5p and ADH4 need to be explored at a deeper level.

# Conclusions

By studying the role and mechanism of the regulation network of LINC01018/hsa-miR-182-5p/ ADH4 axis found above in the occurrence and development tendency of HCC, it is expected to provide new molecules and potential new targets for the prognosis, diagnosis and therapy of HCC[30], so as to reduce the mortality of liver cancer and other cancers and make a new breakthrough in the medical field.

# Abbreviations

BP
Biological Processes
CC
Cell Components
DAVID
the Database for Annotation,Visualization and Integrated Discovery
DEGs
differentially expressed genes
GEO
Gene Expression Omnibus
GEPIA
Gene Expression Profiling Interactive Analysis
GO
Gene Ontology
GTEX
Genotype-Tissue Expression
HCC
Hepatocellular carcinoma
KEGG
Kyoto Encyclopedia of Genes and Genomes
LASSO
Least absolute shrinkage and selection operator
MF
Molecular Functions
TCGA
The Cancer Genome Atlas

# Declarations

## AUTHOR CONTRIBUTIONS

Xiaolong Li participated in the experimental idea and guidance. Liting Wang designed the experiments. Zhenyu Chen and Xiaoling Wang collected the data. Shaolan Jiang and Qiuling Zeng participated in the data analysis and interpretation. Liting Wang, Zhenyu Chen, Xiaoling Wang, Shaolan Jiang and Qiuling Zeng drafted the manuscript. Liting Wang revised the manuscript. Liting Wang, Zhenyu Chen and Xiaoling Wang were the co-first authors. All authors read and approved the final manuscript.


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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.


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## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## CONSENT FOR PUBLICATION

All authors give consent for publication.

## COMPETING INTERESTS

The authors declare that they have no conflict of interest.

## AVAILABILITY OF DATA AND MATERIALS

The datasets used or analyzed in this study are available from the corresponding author.

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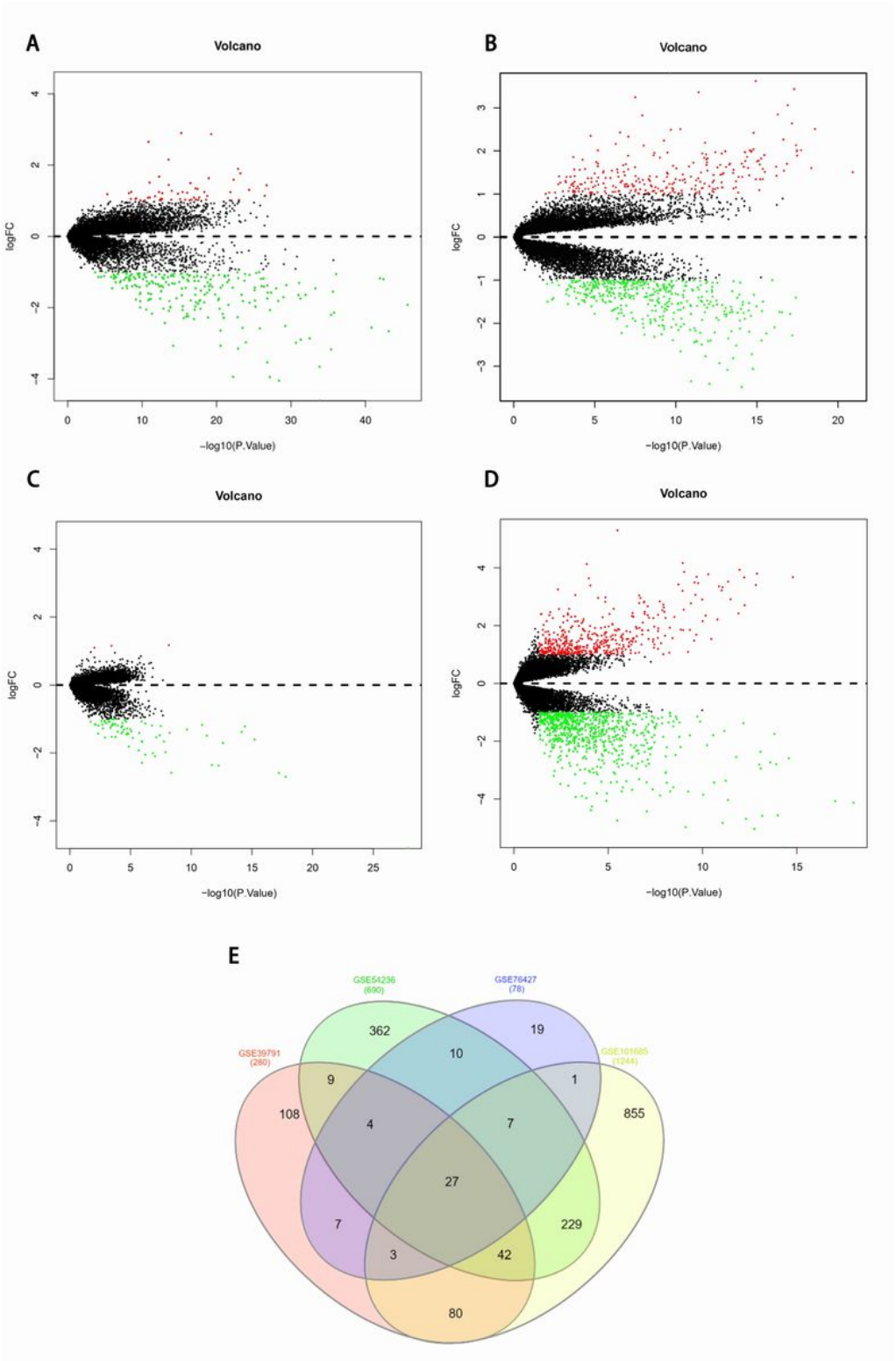
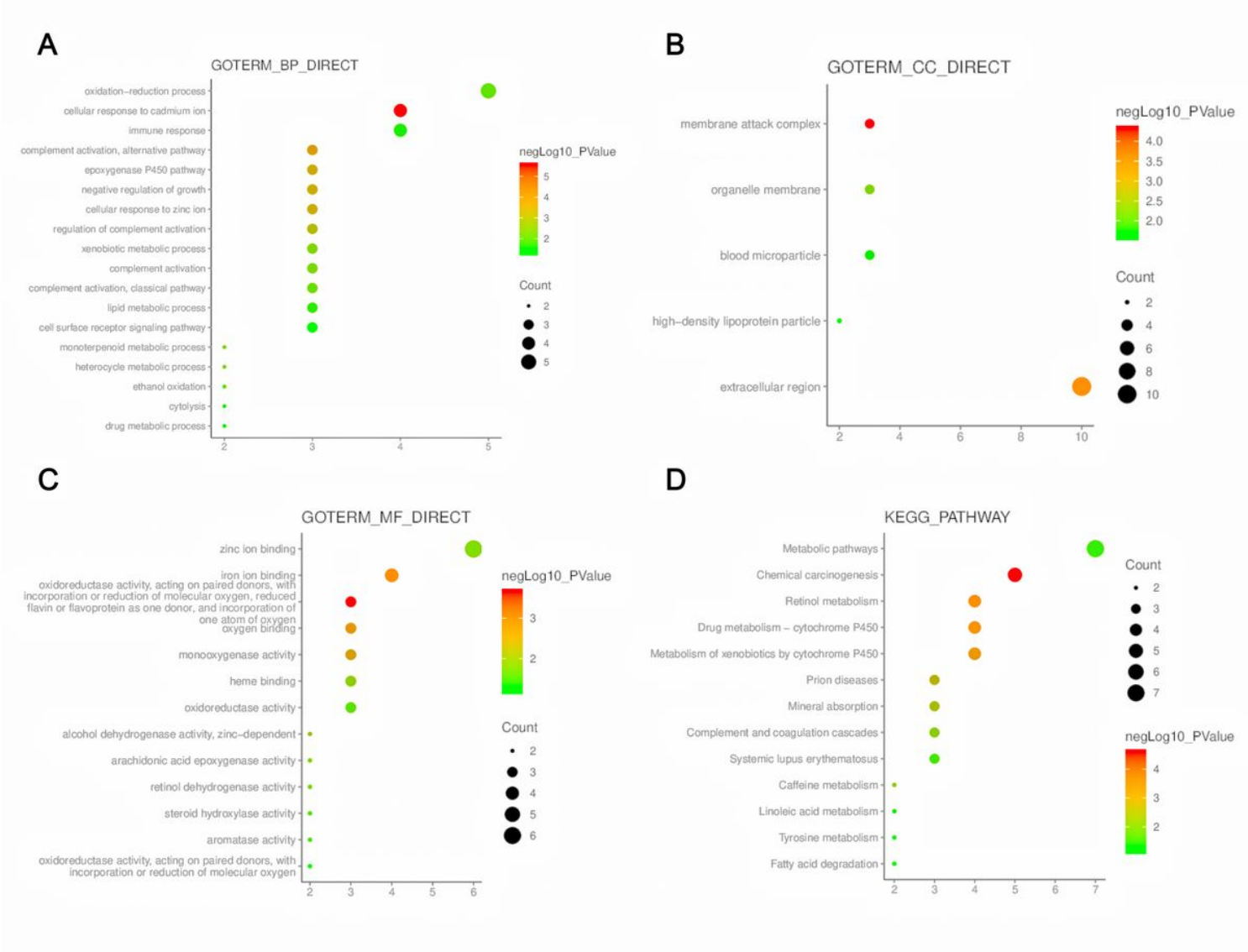
# Figures




## Figure 1

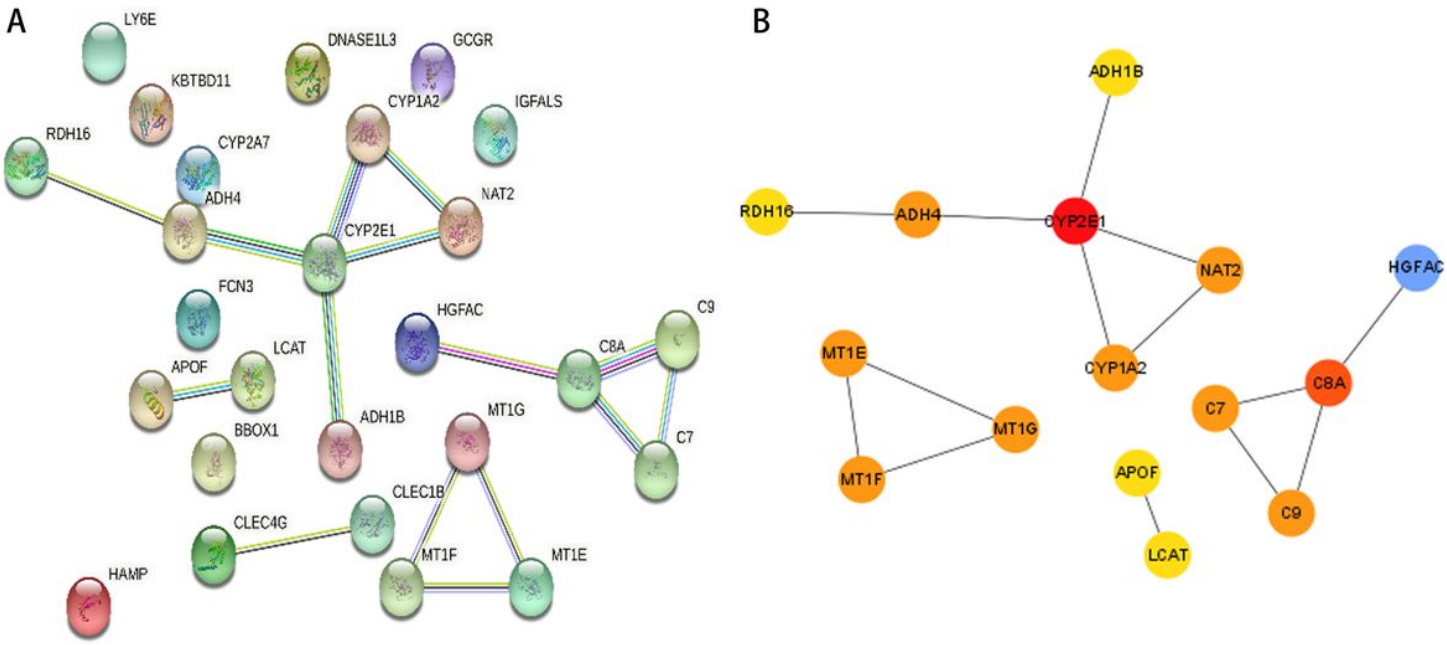
Differential expression of data between four sets of samples. Notes: (A) GSE39791 data, (B) GSE54236 data, (C) GSE76427 data, (D) GSE101685 data. The red points represent upregulated genes screened on the basis of |fold change|>2.0 and a corrected P-value of <0.05. The green points represent down regulation of the expression of genes screened on the basis of |fold change|>2.0 and a corrected P-value

of <0.05. The black points represent genes with no significant difference.(E) Venn diagrams of differentially expressed genes in GSE39791, GSE54236, GSE76427 and GSE101685.



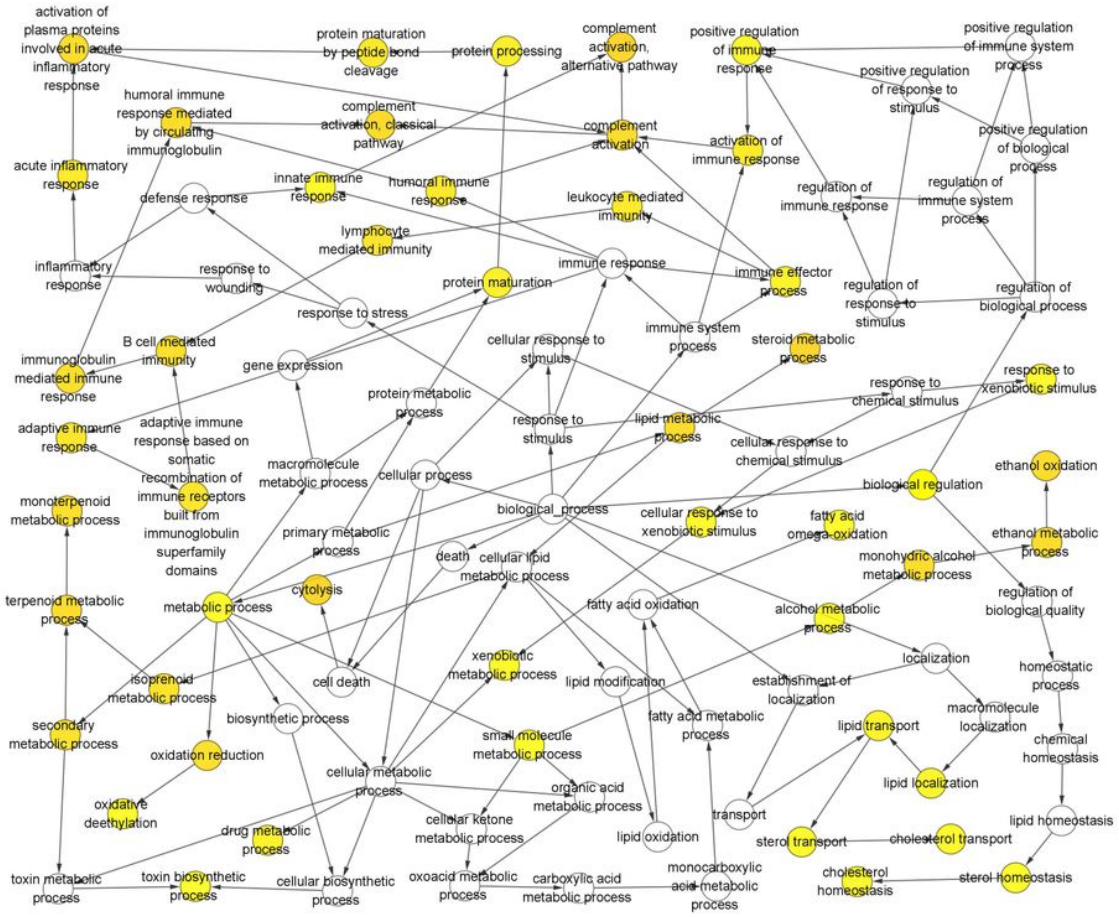

## Figure 2

Functional annotations for important DEGs. Analysis of enriched cell components (CC), biological processes (BP) and molecular functions (MF). GO: Ontology of genes.



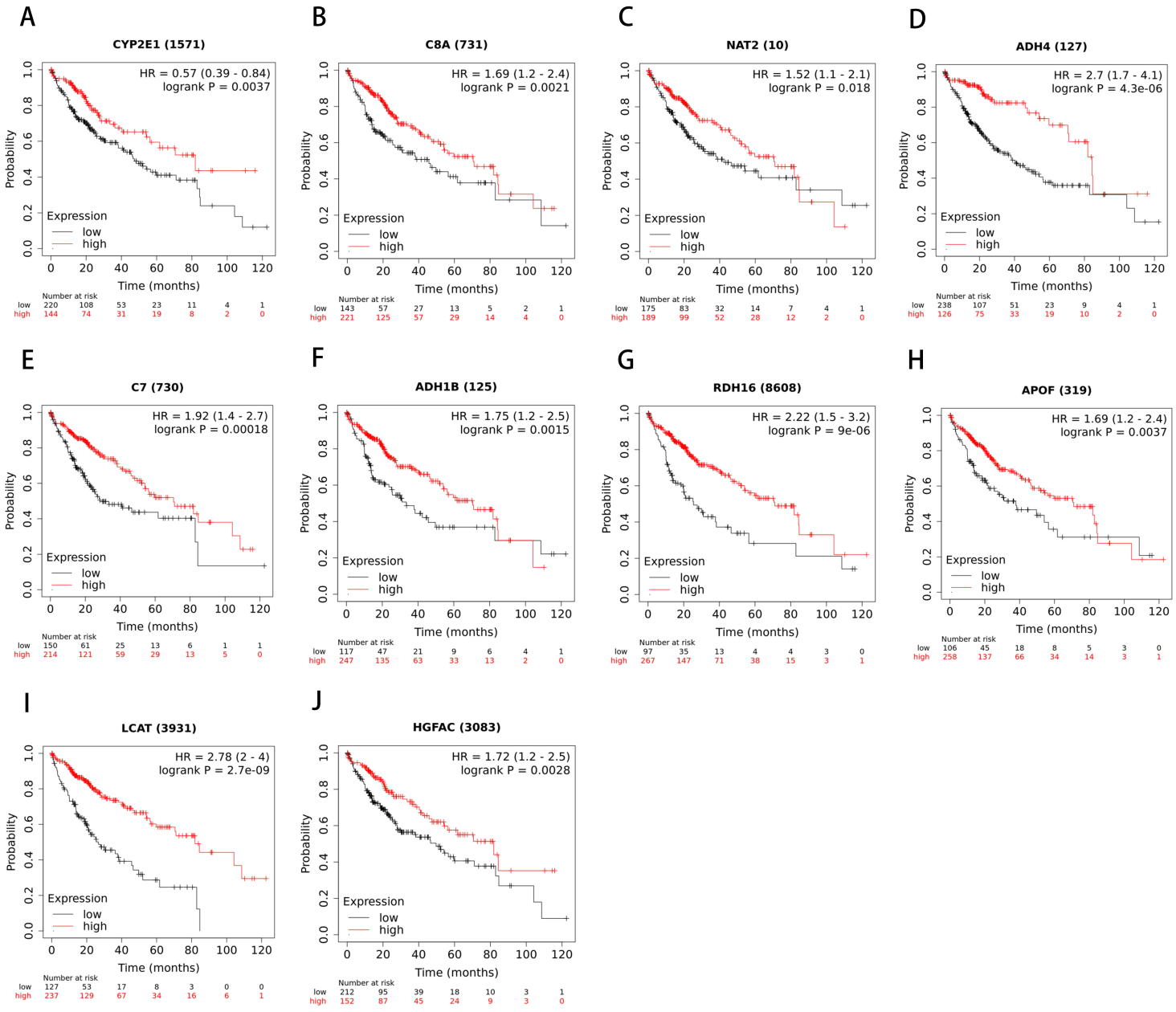

## Figure 3

Identification of the hub gene in the protein interaction (PPI) network. (A) Construct PPI network of important DEGs with STRING. (B) Use Cytoscape's plug-in MCODE to select the most important module from the PPI network.



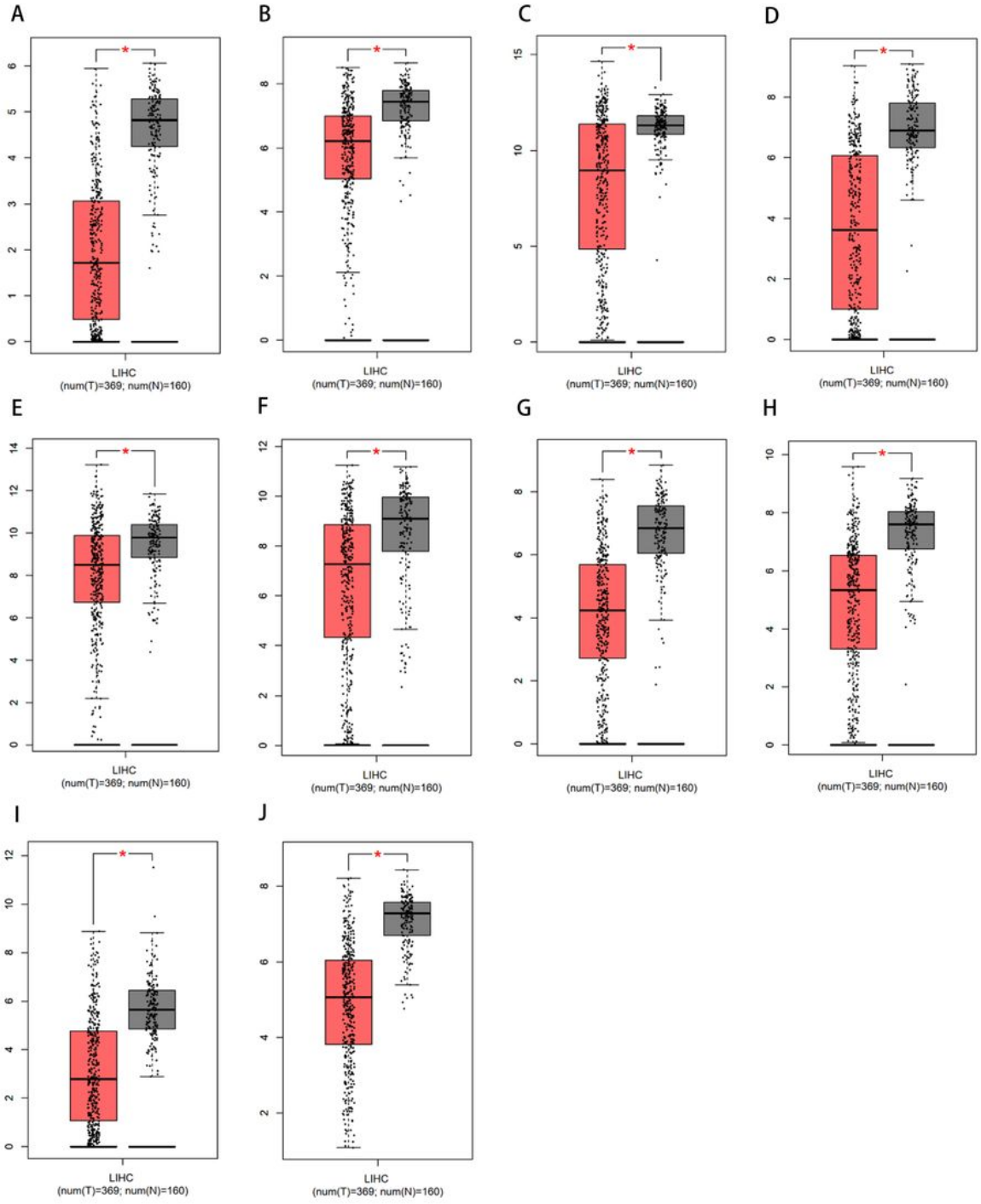

## Figure 4

Biological process analysis of hinge genes. Biological process analysis of hub gene constructed by BiNGO. The color depth of the node refers to the corrected P-value of the ontology. Node size refers to the number of genes involved in the ontology. P<0.01 was statistically significant.




## Figure 5

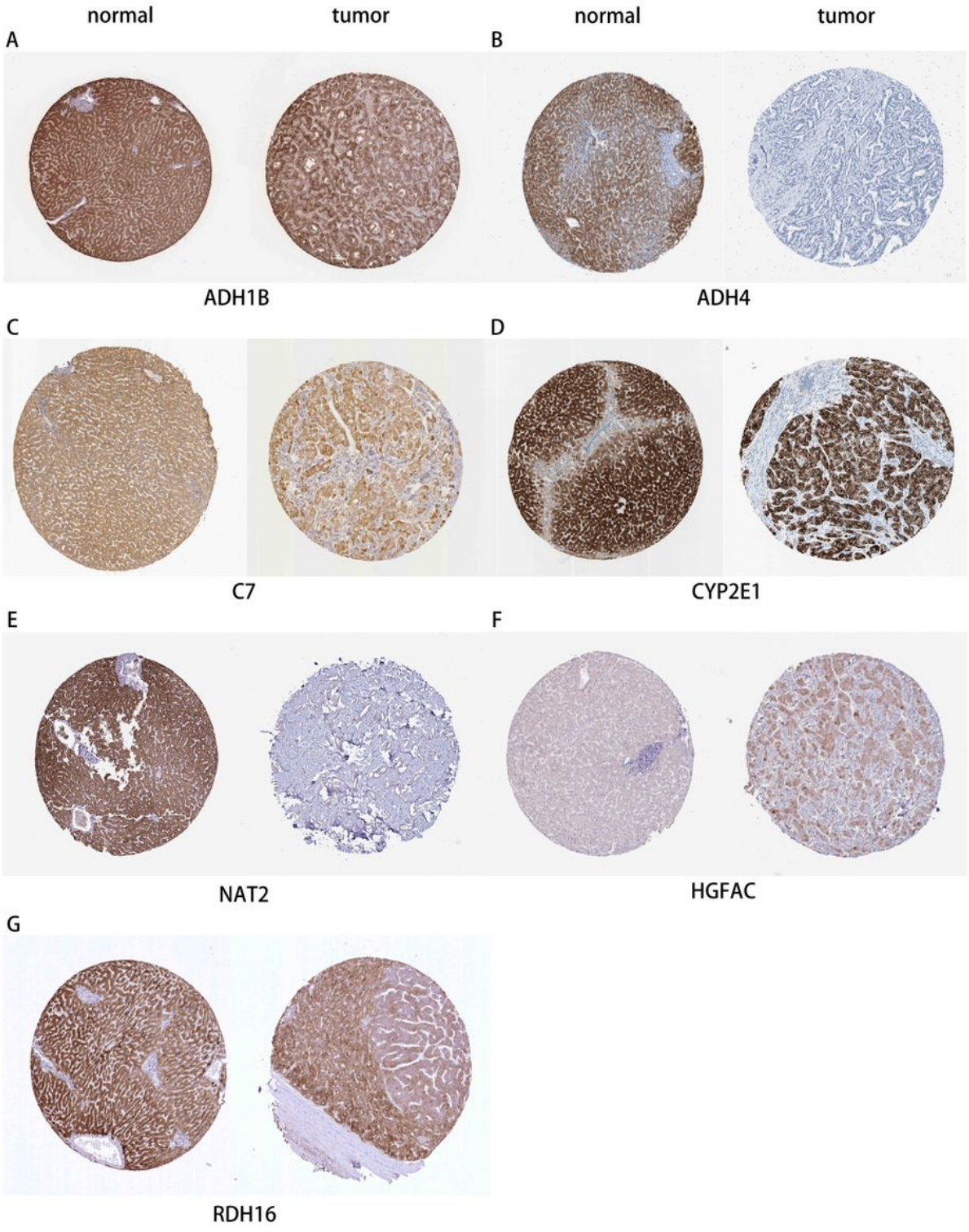
Using the Kaplan-Meier plotter database to analyze the overall survival rate of the hub gene. (A) The prognostic value of CYP2E1 in liver cancer. (B) The prognostic value of C8A in liver cancer. (C) The prognostic value of NAT2 in liver cancer. (D) The prognostic value of ADH4 in liver cancer. (E) The prognostic value of C7 in liver cancer. (F) The prognostic value of ADH1B in liver cancer. (G) The prognostic value of RDH16 in liver cancer. (H) The prognostic value of APOF in liver cancer. (I) The prognostic value of LCAT in liver cancer. (J) The prognostic value of HGFAC in liver cancer. (P<0.05 is statistically significant.




## Figure 6

The expression level of hub gene in tumor tissue and normal tissue by GEPIA. (A) The expression level of NAT2 in liver cancer. (B) The expression level of C8A in liver cancer. (C) The expression level of CYP2E1 in liver cancer. (D) The expression level of HGFAC in liver cancer. (E) The expression level of ADH1B in liver cancer. (F) The expression level of ADH4 in liver cancer. (G) The expression level of APOF in liver cancer. (H) The expression level of RDH16 in liver cancer. (I) The expression level of C7 in liver cancer. (J) The

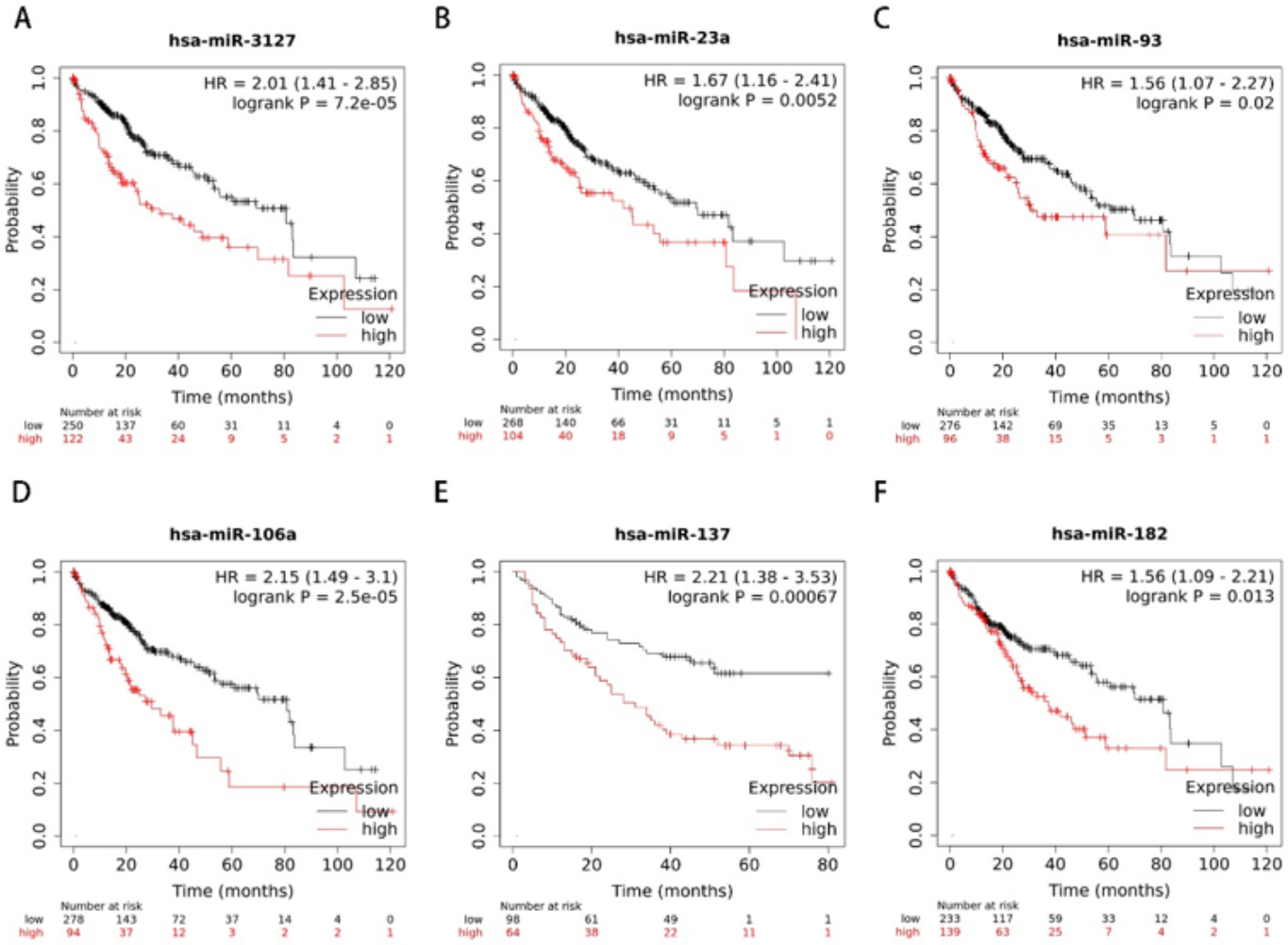
expression level of LCAT in liver cancer. P<0.05 is statistically significant. The Y axis represents the relative expression value log2 (TPM+1).




## Figure 7

Immunohistochemical analysis of hub gene expression levels in HCC tissues and normal tis-sues.(A) Expression level of ADH1B in liver cancer. (B) Expression level of ADH4 in liver cancer. (C) Expression

level of C7 in liver cancer. (D) Expression level of CYP2E1 in liver cancer. (E) Expression level of NAT2 in liver cancer. (F) Expression level of HGFAC in liver cancer. (G) Expression level of RDH16 in liver cancer.

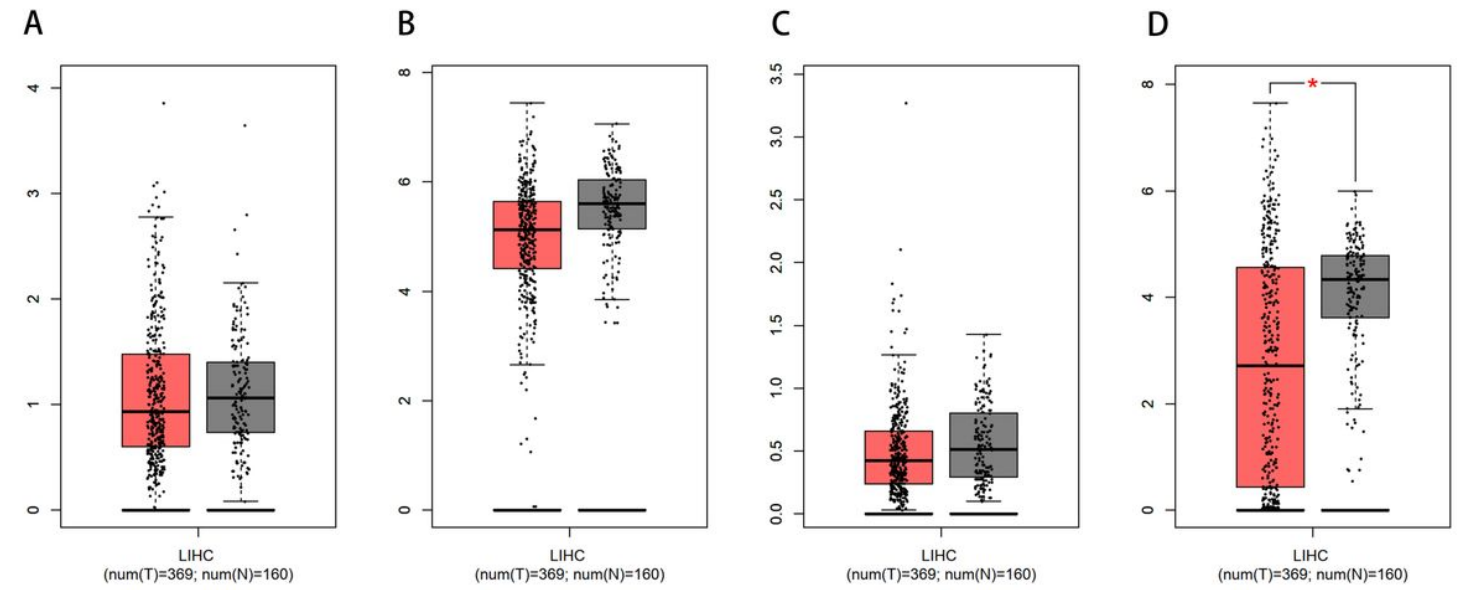



## Figure 8

Combined with miRNA prediction and survival analysis to predicted the upstream miRNA by ADH4 and LCAT. (A) The prognostic value of hSA-Mir-3127-5p in liver cancer. (B) The prognostic value of hSA-Mir-23a-3p in liver cancer. (C) The prognostic value of hSA-Mir-93-5p in liver cancer. (D) The prognostic value of hSA-Mir-106a-5p in liver cancer. (E) The prognostic value of hSA-Mir-137-5p in liver cancer. (F) The prognostic value of hSA-Mir-182-5p in liver cancer.

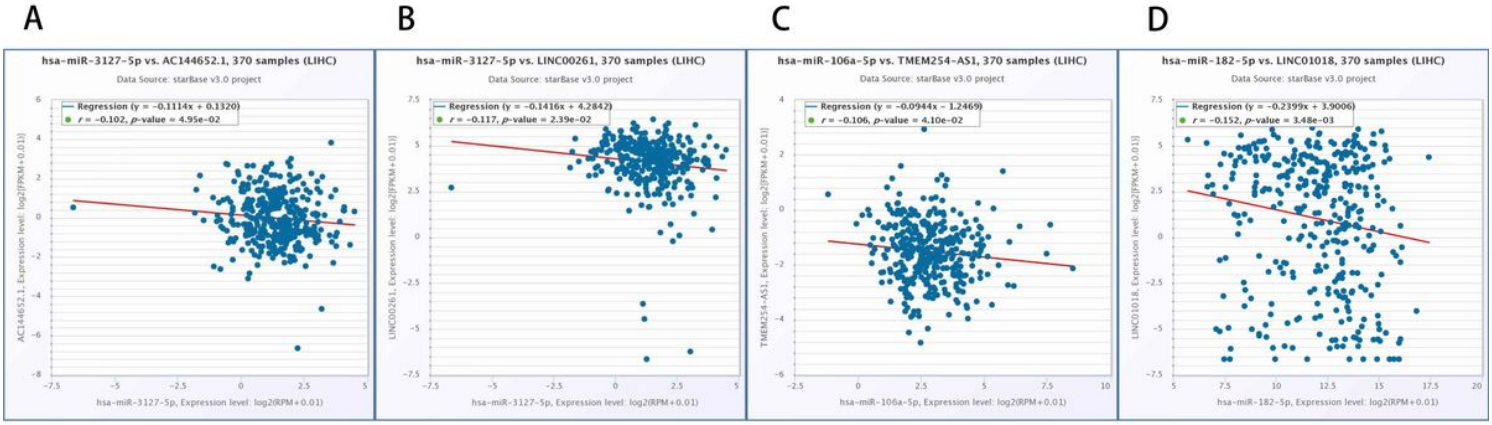

## Figure 9

Expression correlation between miRNA and gene in liver cancer tissue. (A) Expression correlation between hSA-Mir-3127-5p and LCAT in liver cancer tissues. (B) Expression correla-tion between hSA-Mir-23a-3p and ADH4 in liver cancer tissues. (C) Expression correlation between hSA-Mir-93-5p and ADH4 in liver cancer. (D) Expression correlation between HSA-Mir-106a-5p and ADH4 in liver cancer. (E) Expression correlation between hSA-Mir-137-5p and LCAT in liver cancer. (F) Expression correla-tion between hSA-Mir-182-5p and ADH4 in liver cancer.



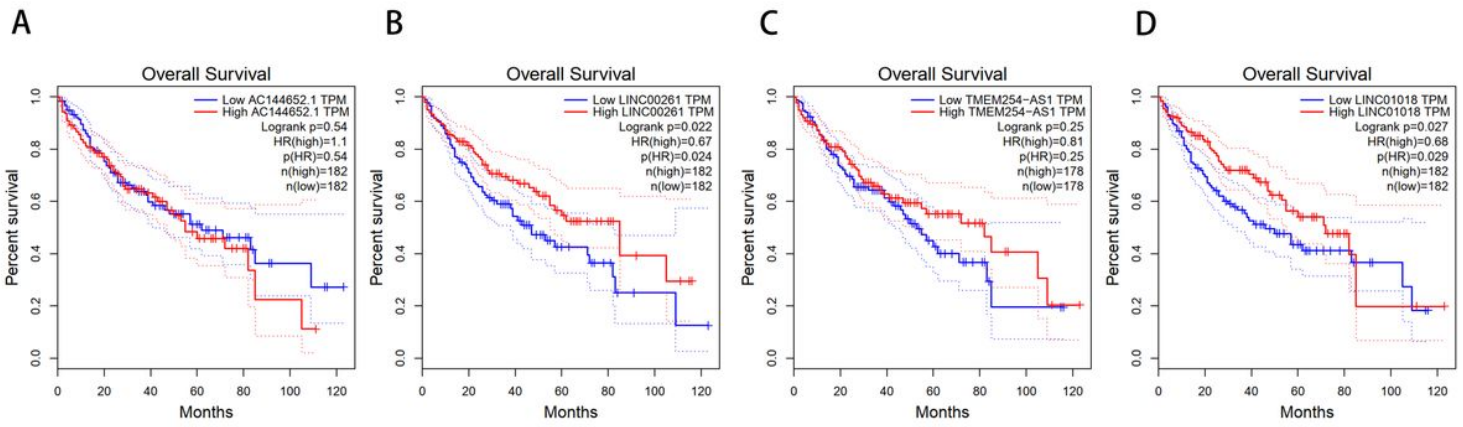

## Figure 10

Expression level of screened lncRNA in liver cancer.(A) The expression level of AC144652.1 in liver cancer. (B) The expression level of LINC00261 in liver cancer. (C) The expression level of TMEM254-AS1 in liver cancer. (D) The expression level of LINC01018 in liver cancer.




## Figure 11

Correlation between the filtered lncRNA and downstream miRNA. (A) Correlation between AC144652.1 and hsa-3127-5p. (B) Correlation between LINC00261 and hsa-miR-3127-5p. (C) Correlation between TMEM254-AS1 and hsa-miR-106a-5p. (D) Correlation between LINC01018 and hsa-miR-182-5p. (P<0.05)




## Figure 12

Prognostic analysis of screened lncrnas. (A) Prognosis of AC144652.1 in liver cancer. (B) Prognosis of LINC00261 in liver cancer. (C) Prognosis of TMEM254-AS1 in liver cancer. (D) Prognosis of LINC01018 in liver cancer. ($p < 0.05$)