Construction of circRNA-based ceRNA Network to Reveal the Role of circRNAs in the Progression and Prognosis of Hepatocellular Carcinoma

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Research

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Abstract

**Background:** Circular RNAs (circRNAs) are now under hot discussion as novel promising bio-markers for patients with hepatocellular carcinoma. The purpose of our study is to identify several competing endogenous RNAs (ceRNAs) networks related to the prognosis and progression of hepatocellular carcinoma, and to further investigate the mechanism of their influence on tumor progression.

**Methods:** First, we obtained gene expression data related to liver cancer from the TCGA database (http://www.portal.gdc.cancer.gov/), including miRNA-seq, RNA-seq and clinical information. A co-expression network was constructed through the WGCNA software package in R software, with the purpose of identifying important microRNAs (miRNAs) and messenger RNAs (mRNAs) related to liver cancer. The DEmRNAs in the key module were analyzed with DAVID (https://david.ncifcrf.gov/summary.jsp) to perform functional enrichment analysis including Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO). The data of miRNA expression and clinical information downloaded from TCGA was utilized for survival analysis to detach the prognostic value of the DEmiRNAs of the key module.

**Results:** 201 DEmiRNAs and 3783 DEmRNAs were finally identified through differential expression analysis. The co-expression networks of DEmiRNA and DEmRNA were constructed by using WGCNA. Further analysis confirmed 4 miRNAs in the most significant module (blue module) were associated with the OS of patients with liver cancer, including hsa-miR-92b-3p, hsa-miR-122-3p, hsa-miR-139-5p and hsa-miR-7850-5p. DAVID was used for functional enrichment analysis of 286 co-expressed mRNAs. The Gene Ontology (GO) analysis results showed that the top enriched GO terms were oxidation-reduction process, extracellular exosome and iron ion binding. In Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, the top 3 enriched terms included metabolic pathways, fatty acid degradation and valine, leucine and isoleucine degradation. In addition, we crossed the miRNA-mRNA interactions prediction results with the differentially expressed and prognostic mRNAs, and found that hsa-miR-92b-3p can be related to cytoplasmic polyadenylation element binding protein 3 (CPEB3) and Acyl-CoA Dehydrogenase Long Chain (ACADL). By overlapping the data of predicted circRNAs by circBank and differentially expressed circRNAs of GSE94508, we screened has_circ_0077210 as the upstream regulatory molecule of hsa-miR-92b-3p. Hsa_circ_0077210/hsa-miR-92b-3p/CPEB3&ACADL were validated in hepatic cell carcinoma (HCC) tissues and human protein atlas (HPA) database.

**Conclusion:** Our research provides a mechanistic elucidation of the unknown ceRNA regulatory network in HCC. Hsa_circ_0077210 might serve as an important biomarker to promote the occurrence and development of HCC.

**Introduction**

Liver cancer is a common malignant tumor worldwide and ranks second among cancer-related deaths [1]. Although great efforts had been made in the prevention and treatment of Hepatocellular carcinoma
(HCC), the incidence and mortality of HCC are still on the rise [2]. The timely diagnosis of liver cancer is of great significance for improving the survival time of patients. For patients with Barcelona Clinic Liver Cancer (BCLC) stage 0 or A, the 5-year survival rate after surgery is 90% and 50–70% respectively [3–5]. Unfortunately, patients with BCLC staging of B, C, or D will not have a survival rate higher than 16% at 5 years after surgery [6]. Based on the current status of diagnosis and treatment of HCC, and it is very urgent to find effective molecular targets for HCC. When patients with HCC could not be diagnosed in the early stage of HCC, there was no effective treatment that could improve the overall survival (OS) of patients.

In fact, protein coding genes account for less than 2% of the entire genome [7]. A large amount of non-coding RNA, for a long time, was considered “transcriptional noise” with little function. However, evidence showed that non-coding RNAs, including microRNAs (miRNA), long-chain non-coding RNAs (lncRNA), and circular RNAs (circRNAs), could play important roles in HCC [8–11]. Pandolfi et al. put forward the hypothesis of competing endogenous RNA (ceRNA), which suggested that mRNA, pseudogenes, and Non-coding RNA (ncRNA) are "crosstalked" through common microRNA response elements (MRE) [7]. Moreover, this hypothesis provided a theoretical basis for mRNA, miRNA and circRNA to construct a regulatory network.

In the past, circRNA was considered as a by-product of abnormal splicing and had little potential to play a role in physiological or pathological processes [12]. However, the role of circRNA under physiological and pathological conditions has been partially explored [13]. Recent studies have shown that the synthesis of circRNA depends on the spliceosome mechanism and is regulated by cis-complementary sequences and protein factors [13]. Although the known functions of circRNA include isolating microRNAs or proteins, regulating transcription and splicing, and translation to produce polypeptides [13]. Above mentioned functions are still little part of the functions of circRNA, and there are a large number of functions related to circRNA that need to be explored.

In this study, we screened differentially expressed miRNAs and mRNAs associated with survival through WGCNA. In addition, we described the expression and lineage of circRNA in HCC, and investigated the mechanisms of ceRNA netework to the development and metastasis of HCC.

**Methods**

**Data acquisition and differential expression screening**

First, we obtained gene expression data related to liver cancer from the TCGA database (http://www.portal.gdc.cancer.gov/), including miRNA-seq, RNA-seq and clinical information. Using the edger R software package, we analyzed the expression data with clinical information and deleted duplicate data, and obtained differentially expressed mRNA and miRNA (DEmRNA and DEmiRNA respectively). Next, |Log 2-fold change| ≥ 1.0 and P-value < 0.05 were used of filter condition to get mRNAs and miRNAs for further analysis. The data obtained from TCGA in the study is publicly available,
and the approval of the local ethics committee is not necessary. Then, we searched HCC related circRNA expression microarray information from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). GSE94508 (data from five primary hepatocellular carcinomas and five matched normal liver tissues) was utilized for further analysis. The Limma software package (version: 3.40.2) can be used to study the differential expression of circRNA (DEcircRNA). We set the threshold for circRNA differential expression screening to $|\text{Record 2 times change}| \geq 1.0$ and $P \text{ value} < 0.05$. Probe sets with circbase ID (http://www.circbase.org) will be included in the research, while probe sets without circbase ID will be deleted.

**WGCNA and identification of the liver cancer carcinogenesis modules**

A co-expression network was constructed using the WGCNA software package in R software, with the purpose of identifying important miRNAs and mRNAs related to liver cancer. The GoodSamplesGenes function can be used to check whether the DEmiRNA and DEmRNA of the data matrix meet the standard. According to this standard, we will exclude unqualified data. Then, in order to ensure a scale-free network, we use the pickSoft-Threshold function to calculate the $\beta$ value (soft threshold power parameter). Next, diagram of tree was established by hierarchical clustering, and the correlation between module eigengenes (MEs) and clinical trait was sum up and used for the filter out the MEs unrelated to the carcinogenesis and progression of HCC. Last, a core-module from all modules based on the highest correlation coefficient for further research.

**Functional enrichment analysis**

The DEmRNAs in the key module were analyzed with DAVID (https://david.ncifcrf.gov/summary.jsp) to perform functional enrichment analysis including Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO). The pathways that had $p < 0.05$ were considered significantly enriched.

**Survival analysis of the miRNAs in the key module and functional pathway enriched**

We utilized data of miRNA expression and clinical information downloaded from TCGA for survival analysis, to detach the prognostic value of the DEmiRNAs of the key module. We screened out DEmiRNAs under the condition of the log-rank $P \text{ value} < 0.05$, and drew survival curves of DEmiRNAs that were significant through the R package "Survminer". The miRPath V3.0 tool was used to enrich pathways of prognosis-related DEmiRNAs.

**Prediction of miRNA–mRNA and circRNA–miRNA interactions**

We selected the first two key modules in WGCNA-DEmiRNAs for survival analysis, and DEmRNAs with a log-rank $P \text{ value} < 0.05$ were considered as potential DEmRNAs. Then, we used the online analysis tools DIANA (http://diana.imis.athena-innovation.gr/) and TargetScan (http://www.targetscan.org/) to predicte
target mRNAs of potential DEmiRNAs. Target genes appearing in both databases were considered to be the predicted results of potential DEmiRNA target genes. Target mRNA that overlaps with potential DEmRNAs was considered as potential mRNA and requires further analysis. We set two screening conditions (binding sites ≥ 3 and Length < 2000), and used the circBank database (http://www.circbank.cn/) to predict potential DEmiRNA target circRNAs. We selected the overlapping part of the target circRNAs from GSE94508 and DEcircRNAs as potential circRNAs to improve the reliability of the analysis. In addition, we retrieved the target circRNA acting on the corresponding DEmiRNA from the circBank database. The part of the target gene that overlapped with DEcircRNAs with mRNA regulatory potential was defined as the key gene. The structure diagram of potential circRNA was detected and downloaded through the online website Cancer-Specific CircRNA (CSCD) (http://gb.whu.edu.cn/CSCD/).

**Construction of circRNA-miRNA-mRNA network**

This regulatory network consisted of downstream regulatory genes of miRNA and circRNA that had potentially regulated miRNA. Then, we used Cytoscape software (version 3.7.2) to construct a visualized circRNA-miRNA-mRNA network.

**Survival analysis of the key genes and Gene Set Enrichment Analysis (GSEA)**

The survival analysis of key genes was constructed by the Kaplan–Meier plotter. Based on the known information of gene characteristics, location and biological functions, we established a database of molecular characteristics by GSEA. We set the cutoff value to p < 0.05 and detected the pathways that were significantly related to the key genes with GSEA analysis.

**RNA extraction, reverse transcription and quantitative PCR (RT-qPCR)**

A total of 15 paired fresh-frozen HCC tissues and normal tissues were obtained from patients diagnosed with clear cell renal cell carcinoma (ccRCC) at the The Affiliated Cancer Hospital of Nanjing Medical University & Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research. Total cellular RNA from tissues was collected with Trizol Regent (Invitrogen). Complementary DNA (cDNA) was reversed from total RNA with PrimeScript™ RT reagent kit (Takara Bio, Inc., Otsu, Japan). Real-time quantitative PCR was utilized to assess the circRNAs expression, which was carried out in triplicate by a Synergy Brands (SYBR) Premix Ex Taq™ kit (Takara Bio) and ABI 7900HT Real-Time PCR system (Applied Biosystems Life Technologies, Foster City, CA, USA). The final results were analyzed through the comparative cycle threshold values (2-ΔΔCt).

**Statistical analysis**

The results are expressed in the form of mean ± standard deviation (SDs). The statistical analysis of the data was performed using the nonparametric tests of SPSS v23.0 software (SPSS Inc., Chicago, Illinois,
USA). To compare two independent groups, we utilized Mann–Whitney U test. P-value < 0.05 of differences were considered statistically significant.

Results

Differential expression screening of miRNA and mRNA in TCGA-LIHC database

We obtained the transcriptome data and clinical information from TCGA-LIHC dataset, including 425 miRNA specimens (50 normal samples and 375 liver cancer samples) and 424 mRNA specimens (50 normal samples and 374 liver cancer samples). Then, DEmiRNAs and DEMRNAs were finally identified. The cutoff threshold of screening was set to |Log 2-fold change| ≥ 1.0 and P-value < 0.05, which revealed 201 and 3783 aberrantly expressed miRNAs and mRNAs, respectively. As shown in Volcano map and Heatmap, the 201 DEmiRNAs included 142 upregulated and 59 downregulated miRNAs (Fig. 1A, 2A), while the 3783 DEMRNAs included 2677 upregulated and 1106 downregulated mRNAs (Fig. 1B, 2B).

WGCNA analysis and identification of liver cancer tumorigenesis modules

To further investigate the carcinogenesis of DEmiRNAs and DEMRNAs, WGCNA package were utilized. Based on the value of soft threshold power $\beta$, the connection between the genes in gene network was found to be consistent with the results of scale-free network distribution. Next, the modules of DEmiRNA and DEMRNA were constructed co-expression networks by using WGCNA. Then, the modules with similar expression profiles were identified by the dynamic tree-cut diagram (Fig. 3A, B and Fig. 4A, B). After analyzing and classifying, 11 modules for DEmiRNAs and 16 modules for DEMRNAs were identified. In view of the heatmap of module-trait relationships (Fig. 3C), we could find that blue module of DEmiRNAs was the highest positive correlation with tumor tissues (correlation coefficient=-0.79, $P = 1e-42$). In addition, the yellow modules of DEMRNAs showed the most significant positive correlation with tumor tissues (correlation coefficient=-8, $P = 4e-94$: Fig. 4C). According to the miRNA and mRNA data of the most significant module among the constructed modules (Table 2,3,4), the correlations between gene significance for normal or tumor and module membership in blue and yellow were verified, respectively (Fig. 3D,E;Figure 4D,E).

Survival analysis and pathway analysis of the miRNA in the most significant module

To further reveal which of the DEmiRNAs were the prognostic value miRNA, we analyzed the association between the expression of all miRNAs in the most significant module (blue module) and over survival (OS) of patients with liver cancer. Then, we found 4 miRNAs associated with the OS of patients with liver cancer, including hsa-miR-92b-3p, hsa-miR-122-3p, hsa-miR-139-5p and hsa-miR-7850-5p. As shown in Fig. 5E-H, hsa-miR-92b-3p was upregulated in the tumor tissues; hsa-miR-122-3p, hsa-miR-139-5p and
hsa-miR-7850-5p were both downregulated in the tumor tissues. Next, we established OS curves of them and found the high expression of hsa-miR-92b-3p and hsa-miR-7850-5p and the low expression of hsa-miR-122-3p and hsa-miR-139-5p had poor survival prognosis (Fig. 5A-D). KEGG pathway analysis based on the 4 miRNAs was carried out using DIANA-miRpath v.3, a web tool. The results revealed that all miRNAs were related to tumour-associated pathways, especially PI3K-Akt signaling pathway, pantothenate CoA biosynthesis and signaling pathways regulating pluripotency of stem cells (Fig. 5I).

**Functional enrichment analysis of the co-expressed mRNAs in the key modules**

In order to fully evaluate the contribution of DEmRNAs in the key modules (yellow and brown modules) to the development and progression of liver cancer, DAVID was used for functional enrichment analysis of 286 co-expressed mRNAs. The Gene Ontology (GO) analysis results showed that: the top 3 enriched biological processes were oxidation-reduction process, metabolic process and lipid metabolic process (Fig. 6A); the top 3 enriched cell components were extracellular exosome, extracellular region and extracellular space (Fig. 6B); top 3 enriched molecular functions were iron ion binding, heme binding and electron carrier activity (Fig. 6C). In Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, the top 3 enriched terms included metabolic pathways, fatty acid degradation and valine, leucine and isoleucine degradation (Fig. 6D).

**Screen of miRNA-mRNA interactions and pathway analysis of target mRNAs**

First, we performed overall survival (OS) analysis and plotted survival curves of the top 2 significant modules in WGCNA-mRNA (Figure S1). Second, Targetsca and DIANA was utilized to predict the target mRNAs of the four differentially expressed and prognostic miRNAs, which might regulate in the downstream pathway. Then, we crossed the prediction results with the differentially expressed and prognostic mRNAs, and found that hsa-miR-92b-3p can be related to CPEB3 and ACADL (Fig. 7A). The expression of CPEB3 and ACADL were downregulated in tumor tissues (Fig. 7C,E). Base on the clinical information, the high expression of CEPE3 and ACADL predicted better OS (Fig. 7B,D). The result was consistent with the expression trend. In fact, the low expression of CPEB3 and ACADL could be associated with higher tumor grade, but could not be associated with with gender, tumor stage or TNM (Figure S2). Moreover, to understand the functional pathway of CPEB3 and ACADL, Gene Set Enrichment Analysis (GSEA) was performed, respectively. Three pathways including G2M checkpoint, mtorc1 signaling and cell cycle, showed a positive correlation with low CPEB3 expression (Fig. 8A-C). Meanwhile, the GSEA of ACADL showed 3 the most significant enriched pathways, including myc targets v1, myc targets v2 and wnt beta catenin signaling (Fig. 8D-F). In addition, we used web tool TIMER to analyze the correlation between expression levels of CPEB3 and ACADL and immune cells. The results confirmed that CPEB3 and ACADL were significantly related to tumor immunity: CPEB3 was related to the content of B cells and neutrophil; ACADL was related to the infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophage and dendritic cell (Figure S3A,B).
Exploring the circRNA that regulates hsa-miR-92b-3p

According to above results, hsa-miR-92b-3p had the potential to regulate CPEB3 and ACADL. Base on the hypothesis of ceRNA regulation, there may be potential circRNA regulating hsa-miR-92b-3p. Therefore, we searched for possible upstream circRNA of hsa-miR-92b-3p through circbank database and GEO liver cancer dataset GSE94508. First, we explored the differentially expressed circRNAs between liver cancer and normal tissues. The cutoff threshold of screening was set to |Log 2-fold change| ≥ 1.0 and Adjust P-value < 0.05. As shown in Volcano map and Heatmap, the 534 DEciRNAs included 199 upregulated and 335 downregulated circRNAs (Fig. 9A, B). Through the circBank database, setting the binding site ≥ 3 and length ≥ 2000 bp as the screening conditions, we screened 311 potential circRNAs that might regulate hsa-miR-92b-3p (Fig. 9C). By overlapping the data of predicted circRNAs by circBank and differentially expressed circRNAs of GSE94508, we screened has_circ_0077210 as the upstream regulatory molecule of hsa-miR-92b-3p (Fig. 9D). Then, the structure and information of has_circ_0077210 were explored by CSCD and circBank database (Fig. 9E) (Table 5).

Construction of CeRNA regulatory axis and expression validation by qRT-PCR

By using cytoscape, we mapped the regulatory axis of has_circ_0077210/ hsa-miR-92b-3p/CPEB3, ACADL (Fig. 9F). Then, the relative expression levels of has_circ_0077210, hsa-miR-92b-3p, CPEB3 and ACADL were different between HCC tissue and normal tissue (P < 0.05) (Fig. 10). Additionally, The Human Protein Atlas (HPA) database showed that ACADL expression level was significantly lower in HCC tissue than normal liver tissues (Supplement Fig. 4).

Discussion

In developing countries, hepatocellular carcinoma (HCC) is the most common pathological type of liver cancer. Most HCC patients could not prolong their life due to tumor metastasis or recurrence, and the specific mechanism remains unclear. Although it has been proven that radical liver cancer resection is an effective treatment for early HCC, patients could not diagnose in time, most patients still cannot be cured. The mechanism of HCC is mainly discussed from the genetic level, and the molecular mechanism is of great significance for the treatment of HCC. Non-coding RNA (ncRNA), including Inc RNA, microRNA and circRNA, is transcribed from the genome without being translated into protein. NcRNA can control gene expression at various levels, including epigenetic modification [14, 15], transcription[15], RNA splicing[16, 17], scaffold assembly[17], etc. CircRNAs has tissue-specific expression pattern and has potential to be biomarker, so it could be used as clinical diagnosis and prognostic indicators. Due to the role of circRNA in HCC has not been fully elucidated, the bioinformation analysis of circRNAs mediate ceRNA regulatory network.

Based on public databases (GEO and TCGA), we compared the differentially expressed genes in HCC. Based on multiple databases, a ceRNA axis (circRNA / miRNA / mRNA) in HCC was established.
Additionally, functional analysis was performed to infer the biological functions of key genes in HCC. Finally, the key tumor suppression axis (hsa_circ_0077210/hsa-miR-92b-3p/CPEB3 and ACADL) was constructed. Our data provided a new way of mining circRNA related to the ceRNA axis in HCC, and identified potential prognostic and therapeutic targets.

In recent years, non-coding RNA has received extensive attention. With the ceRNA hypothesis has been put forward, the role of circRNA in the occurrence of HCC has more motivation to be detached. Previous studies have shown that circRNA has transcriptional disorder in many cancers, including HCC, which might be related to tumor proliferation and metastasis. Zheng et al. found that by inhibiting the expression of circSEPT9, the proliferation, migration and invasion of triple-negative breast cancer (TNBC) could be inhibited, and the apoptosis and autophagy of TNBC cells could be induced [18]. The result showed that circRNA played significant role in occurrence and development of refractory breast cancer. Yang et al. verified the RNA expression level of circPTK2 in colon cancer and normal colon tissues, and verified that the expression content of circPTK2 is related to the clinical stage of colon cancer patients, and in vitro and PDTX models, verified that the increase in circPTK2 expression can be Promote the metastasis of colon cancer [19]. The results of Chen et al. confirmed that circSnx5 could act as a miR-544 sponge to reduce Suppressors of cytokine signaling 1 (SOCS1) target inhibition and inhibit the nuclear transport of PU.1, and regulate dendritic cell (DC) activation and function [20].

MiRNA, belong to non-coding RNA, could affect the pathological process of various cancers by affecting the expression level of oncogenes or tumor suppressor genes. Fan et al. investigated the association between serum microRNA and checkpoint inhibitor response in non-small cell lung cancer (NSCLC), and the results showed that the statistically significant improvement in patient progression-free survival (PFS) was correlated with the 10 highly expressed miRNA patterns And in NSCLC patients treated with anti-PD1 drugs, changes in circulating miRNAs are related to the effects of anti-PD1 drugs and patient prognosis [21]. The results showed that the transcription level of microRNAs was related to the tolerance and effectiveness of immunotherapy. Research of Cheray et al. showed that many members of miRNA contain 5mc, and some fragments of cytosine residues could methylate miRNA, and inhibit the formation of miRNA/mRNA duplexes, resulting in the loss of the duplex's function of suppressing gene expression, and further led to the occurrence of glioblastoma multiforme (GBM) [22]. Explaining the interaction mechanism of miRNA and exosomes is also a hot topic. Shi et al. confirmed that the upregulation of miR-520b in exosomes might hinder the proliferation, migration and invasion of pancreatic cancer (PC) cells and induce apoptosis of PC cells by targeting ZNF367 [23]. In fact, the role of hsa-miR-92b-3p had been partly detected in many cancers. For example, wang et al. confirmed that upregulated hsa-miR-92b-3p could weaken the G0/G1 phase and induce migration and invasion of esophageal squamous cell carcinoma (ESCC) cells in vitro by directly targeting Kruppel-like factor-4 (KLF4) and Data Science and Cybersecurity Center 2 (DSC2) [24]. However, the relationship between has-miR-92b-3p and HCC and its regulatory mechanism have not been elucidated.

We predicted CPEB3 and ACADL through the downstream gene prediction of hsa-miR-92b-3p with a series of screening conditions. In fact, CPEB3 and ACADL might participate in the several progress of
HCC. Tang et al. found that CPEB3 was involved in the regulation of mir-452-3p in HCC, and down-regulating the expression of CPEB3 could induce autophagy of HCC cells and increase the migration, invasion and proliferation abilities of HCC cells [25]. Zhao et al. found that in liver cancer, the expression levels of ACADL and YAP are negatively correlated [26]. Huang et al. confirmed that the lack of ACADL can promote the occurrence of HCC by reducing the expression level of Phosphatase and tensin homology deleted on chromosome ten (PTEN) [27]. It had been demonstrated that YAP suppresses PTEN via regulating miR-29 [28]. Therefore, ACADL may inhibit the occurrence of liver cancer through YAP/PTEN signaling pathway.

Based on the GSEA analysis of CPEB3 and ACADL, a visual overview of the mechanisms by which CPEB3 and ACADL regulate HCC development. The results show that CPEB3 and ACADL can actively regulate the cell cycle, G2M checkpoint, mammalian target of rapamycin 1 (mTORC1) signaling, myc targets v1, myc targets v2 and wnt beta catenin signaling. In the background of HCC, the downstream of mTORC1 includes (S6K1, also known as p70S6 kinase) S6K1, rpS6, 4E-BP1 and epithelial-mesenchymal transition (EMT) processes [29]. The depletion of S6K1 could be followed by many immune responses, such as the activation of major histocompatibility complex major histocompatibility complex (MHC) I cell surface receptor members, and the fluctuation of antigen processing and interferon signaling. Discoveries indicated that S6K1 inhibitors with bevacizumab (anti-VEGF antibody) had potential to modulate the immune response against HCC and immunotherapy methods for HCC [30, 31]. O-GlcNAc transferase (OGT) regulated macrophage inflammation by suppressing S6K1 phosphorylation [32]. In addition, IL-2, IL-4, IFN-γ or TNF-α, in tumor B lymphoid cells, could up-regulate S6K1 signaling to enhance cell viability stimulated by B-cell activating factor of the TNF family (BAFF) [33]. CPEB3 might inhibit the occurrence of HCC by regulating the cell cycle and enhancing the phosphorylation of S6K1 to activate the mTORC1 signaling pathway. Because, the expression level of ACADL was also related to the various immune cells, the hsa_circ_0077210—hsa-miR-92b-3p—CPEB3/ACADL regulation axis might be related to the immune regulation process of HCC.

There are some limitations in our research. First, our ceRNA regulatory axis was based on bioinformatics analysis; the circRNA / miRNA/mRNA regulatory network needs to be confirmed in vivo and in vivo. Second, the expression level of hsa_circ_0077210 needs to be verified in a large sample of RNA samples from HCC patients, and that hsa_circ_0077210 transcription level is related to prognosis. Third, our sample size limited us to univariate and multivariate analysis based on the pathological data of patients.

Conclusion

Our research provides a mechanistic elucidation of the unknown ceRNA regulatory network in HCC. Multivariate analysis showed that CPEB3 and ACADL are independent prognostic factors, suggesting that hsa_circ_0077210—hsa-miR-92b-3p—CPEB3/ACADL axis might play a promoting role in the occurrence and metastasis of HCC. Therefore, hsa_circ_0077210 might become an important biomarker to promote the occurrence and development of HCC.
List Of Abbreviations

Kyoto Encyclopedia of Genes and Genomes (KEGG)
gene ontology (GO)
HCC (hepatic cell carcinoma)
Clinic Liver Cancer (BCLC)
overall survival (OS)
microRNAs (miRNA)
long-chain non-coding RNAs (IncRNA)
circular RNAs (circRNAs)
Non-coding RNA (ncRNA)
competing endogenous RNA (ceRNA)
microRNA response elements (MRE)
module eigengenes (MEs)
Gene Set Enrichment Analysis (GSEA)
reverse transcription and quantitative PCR (RT-qPCR)
clear cell renal cell carcinoma (ccRCC)
standard deviation (SDs)
messenger RNAs (mRNAs)
Acyl-CoA Dehydrogenase Long Chain (ACADL)
polyadenylation element binding protein 3 (CPEB3)
human protein atlas (HPA)
Synergy Brands (SYBR)
triple-negative breast cancer (TNBC)
dendritic cell (DC)
Suppressors of cytokine signaling 1 (SOCS1)
non-small cell lung cancer (NSCLC)
progression-free survival (PFS)
glioblastoma multiforme (GBM)
pancreatic cancer (PC)
esophageal squamous cell carcinoma (ESCC)
Kruppel-like factor-4 (KLF4)
Data Science and Cybersecurity Center 2 (DSC2)
Phosphatase and tensin homology deleted on chromosome ten (PTEN)
mammalian target of rapamycin 1 (mTORC1)
epithelial-mesenchymal transition (EMT)
major histocompatibility complex (MHC)
O-GlcNAc transferase (OGT)
B-cell activating factor of the TNF family (BAFF)

**Declarations**

**Ethics approval and consent to participate**

All the patients provided written informed consent, the protocol was approved by ethical committee of The Affiliated Cancer Hospital of Nanjing Medical University & Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research.

**Consent for publication**

Not applicable.

**Availability of supporting data**

All data generated or analysed during this study are included in this published article and its supplementary information files.

**Competing interests**
The authors declare no competing interests.

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None.

Authors’ contributions

Lin Chen and Jincheng Wang designed this work. Rong Deng and Xiaohan Cui performed the experiment and wrote the manuscript. Yuxiang Dong and Yanqiu Tang performed the bioinformatics analysis. Xuewen Cao and Shuyu Wang performed the pathology experiment and data review. All authors have read and approved the manuscript.

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Tables
Table 1
22 survival-related genes in brown and yellow module.

| gene   | KM(P-value)       |
|--------|-------------------|
| N4BP2L1| 0.0231917680013911|
| C6     | 0.0324979505945558|
| DMGDH  | 0.00345549731847261|
| ACADL  | 0.0458976763753126|
| CYP7A1 | 0.015599256837466  |
| MANF   | 0.0354210475561331 |
| SARDH  | 0.0458207466346884 |
| TUBE1  | 0.0171553602956993 |
| ANXA10 | 0.0281889814075095 |
| SLC38A4| 0.0209045132009409 |
| C8B    | 0.0298561967458564 |
| LPA    | 0.0172195797768141 |
| MAT1A  | 0.0461625356948938 |
| ADH4   | 0.00572238509386069|
| SLC10A1| 0.025877998862526  |
| FNIP2  | 0.0474647059410264 |
| CYP4F2 | 0.0298914997690577 |
| TTC36  | 0.0224862889024342 |
| ADRA1A | 0.0466515861358356 |
| GYS2   | 0.0334809963912731 |
| ABAT   | 0.0259103935446722 |
| CPEB3  | 0.0403726764983748 |
Table 2
46 hub miRNAs in blue module.

| miRNA       | R      | P.value   |
|-------------|--------|-----------|
| hsa-miR-142-5p | 0.813905504 | 6.40E-47  |
| hsa-miR-7850-5p | 0.312758801 | 9.50E-06  |
| hsa-let-7c-3p  | 0.741518906 | 6.09E-35  |
| hsa-miR-6502-5p | 0.68167305  | 1.02E-27  |
| hsa-miR-3614-5p | 0.547438163 | 1.75E-16  |
| hsa-miR-4800-3p | 0.560450155 | 2.35E-17  |
| hsa-miR-130a-3p | 0.853166155 | 7.03E-56  |
| hsa-miR-490-3p  | 0.779091489 | 1.36E-40  |
| hsa-let-7c-5p  | 0.786862206 | 6.69E-42  |
| hsa-miR-4536-3p | 0.596148064 | 5.88E-20  |
| hsa-miR-130a-5p | 0.641736094 | 8.67E-24  |
| hsa-miR-1294    | 0.421849887 | 9.96E-10  |
| hsa-miR-122-3p  | 0.728562531 | 3.24E-33  |
| hsa-miR-335-5p  | 0.682405218 | 8.50E-28  |
| hsa-miR-4683    | 0.574090758 | 2.59E-18  |
| hsa-miR-6503-5p | 0.756774998 | 4.12E-37  |
| hsa-miR-4442    | 0.607451638 | 7.53E-21  |
| hsa-miR-199a-3p | 0.89272708  | 4.82E-68  |
| hsa-miR-551a    | 0.614365541 | 2.05E-21  |
| hsa-miR-144-3p  | 0.622927727 | 3.94E-22  |
| hsa-miR-133b    | 0.588198749 | 2.38E-19  |
| hsa-miR-33b-3p  | 0.74911194  | 5.30E-36  |
| hsa-miR-424-3p  | 0.87924781  | 2.01E-63  |
| hsa-miR-199b-3p | 0.892961816 | 3.96E-68  |
| hsa-miR-1248    | 0.726495132 | 5.99E-33  |
| hsa-miR-139-5p  | 0.849066682 | 7.92E-55  |
| hsa-miR-4454    | 0.491820694 | 3.77E-13  |
| miRNA          | R         | P.value     |
|----------------|-----------|-------------|
| hsa-miR-92b-3p | 0.265364143 | 0.000191709 |
| hsa-miR-1258   | 0.802454172 | 1.06E-44    |
| hsa-miR-139-3p | 0.91885048  | 4.62E-79    |
| hsa-miR-4686   | 0.566095443 | 9.54E-18    |
| hsa-miR-4791   | 0.7146987  | 1.78E-31    |
| hsa-miR-542-5p | 0.806839196 | 1.56E-45    |
| hsa-miR-10a-3p | 0.848676332 | 9.94E-55    |
| hsa-miR-214-3p | 0.86917635  | 2.55E-60    |
| hsa-miR-503-3p | 0.727835823 | 4.02E-33    |
| hsa-miR-195-5p | 0.832317105 | 7.85E-51    |
| hsa-miR-150-3p | 0.655455143 | 4.52E-25    |
| hsa-miR-4685-3p| 0.61011895  | 4.58E-21    |
| hsa-miR-4751   | 0.311563079 | 1.03E-05    |
| hsa-miR-450a-1-3p | 0.44889996 | 5.85E-11  |
| hsa-miR-101-3p | 0.728862754 | 2.96E-33    |
| hsa-miR-511-5p | 0.796661565 | 1.24E-43    |
| hsa-miR-424-5p | 0.892708265 | 4.90E-68    |
| hsa-miR-450a-5p| 0.851068711 | 2.45E-55    |
| hsa-miR-33b-5p | 0.771187957 | 2.58E-39    |
Table 3
119 hub genes in yellow module.

| gene       | R         | P value   |
|------------|-----------|-----------|
| VNN1       | 0.269170352 | 1.80E-08  |
| ECM1       | 0.791692936 | 2.37E-92  |
| TRIB1      | 0.64254245  | 9.64E-51  |
| INS-IGF2   | 0.667195699 | 6.09E-56  |
| DUSP6      | 0.582034823 | 8.27E-40  |
| IER2       | 0.451035694 | 1.23E-22  |
| ADAMTS13   | 0.8131215   | 3.20E-101 |
| LDLR       | 0.484532754 | 2.40E-26  |
| SLC38A2    | 0.603124373 | 2.35E-43  |
| TMEM150B   | -0.30103768 | 2.49E-10  |
| DBH        | 0.649376975 | 3.90E-52  |
| ATF3       | 0.58838342  | 7.53E-41  |
| CLEC4M     | 0.818714181 | 1.00E-103 |
| ADGRG7     | 0.440741125 | 1.41E-21  |
| RCAN1      | 0.691037223 | 1.82E-61  |
| CFP        | 0.793358869 | 5.29E-93  |
| CCL23      | 0.653902678 | 4.45E-53  |
| MAFF       | 0.48561677  | 1.79E-26  |
| PZP        | 0.534926631 | 9.33E-33  |
| COL5A3     | -0.225659085 | 2.68E-06 |
| SERTAD1    | 0.57143375  | 4.02E-38  |
| DTX1       | 0.34683543  | 1.98E-13  |
| SERPINB8   | 0.50327664  | 1.32E-28  |
| SERPINE1   | 0.566790797 | 2.11E-37  |
| TMEM45B    | -0.274509652 | 9.11E-09 |
| FAM46A     | 0.44957588  | 1.74E-22  |
| ID1        | 0.449160176 | 1.93E-22  |
| gene      | R        | Pvalue      |
|-----------|----------|-------------|
| SOCS3     | 0.610388687 | 1.22E-44    |
| KLF10     | 0.48518018 | 2.02E-26    |
| PDE7B     | 0.29284786 | 7.87E-10    |
| CLEC4G    | 0.822865538 | 1.21E-105   |
| C21orf91  | 0.545000083 | 3.59E-34    |
| OIT3      | 0.816708843 | 8.10E-103   |
| CSRNP1    | 0.832384843 | 3.13E-110   |
| LY6E      | 0.322793094 | 9.74E-12    |
| CCDC187   | -0.14534264 | 0.002700702 |
| ZFP36     | 0.76913861  | 4.44E-84    |
| CEACAM20  | -0.076390597 | 0.116268439 |
| PIM1      | 0.328972504 | 3.70E-12    |
| NR4A2     | 0.324536299 | 7.43E-12    |
| EGR2      | 0.536334399 | 5.95E-33    |
| ADRA2B    | 0.535168477 | 8.64E-33    |
| DBNDD1    | -0.185335312 | 0.000123876 |
| NAT2      | 0.620110594 | 2.07E-46    |
| DUSP1     | 0.517029422 | 2.34E-30    |
| GDF2      | 0.850398391 | 9.19E-120   |
| CLEC1B    | 0.819105653 | 6.64E-104   |
| IL18R1    | 0.462308001 | 7.69E-24    |
| SPRY2     | 0.584164399 | 3.72E-40    |
| CDC37L1   | 0.606865161 | 5.18E-44    |
| NR4A3     | 0.56864658  | 1.09E-37    |
| SLC51B    | -0.12846582 | 0.008086268 |
| SLC19A3   | 0.230214902 | 1.66E-06    |
| AQP3      | 0.432050471 | 1.04E-20    |
| FCN3      | 0.792712474 | 9.48E-93    |
| gene     | R         | Pvalue     |
|----------|-----------|------------|
| TIMD4    | 0.534066843 | 1.23E-32   |
| VIPR1    | 0.804149521 | 2.25E-97   |
| KLF6     | 0.469962162 | 1.11E-24   |
| CETP     | 0.540933311 | 1.35E-33   |
| CCDC71L  | 0.538778739 | 2.72E-33   |
| NPM2     | -0.190604681 | 7.83E-05   |
| KBTBD11  | 0.496911606 | 8.00E-28   |
| GADD45B  | 0.62695455  | 1.08E-47   |
| TMPRSS2  | 0.365575792 | 7.47E-15   |
| ANGPTL6  | 0.819506348 | 4.35E-104  |
| CLTCL1   | -0.219055905 | 5.30E-06   |
| CCL14    | 0.537722776 | 3.82E-33   |
| CD5L     | 0.57336532  | 2.00E-38   |
| PLSCR4   | 0.645640398 | 2.28E-51   |
| ZC2HC1C  | 0.453919031 | 6.10E-23   |
| IGFBP3   | 0.457949529 | 2.27E-23   |
| FCN2     | 0.865905877 | 4.92E-129  |
| NFIL3    | 0.550263281 | 6.25E-35   |
| RBP7     | -0.328462291 | 4.01E-12   |
| TDGF1    | -0.173472526 | 0.000332308 |
| SGMS2    | 0.511597054 | 1.18E-29   |
| ETS2     | 0.683916367 | 9.22E-60   |
| BCO2     | 0.676702259 | 4.40E-58   |
| LIFR     | 0.780314867 | 4.69E-88   |
| RIPK4    | 0.478697807 | 1.14E-25   |
| CLDN15   | -0.304453501 | 1.52E-10   |
| PHLDA1   | 0.728176794 | 3.10E-71   |
| COLEC10  | 0.877611439 | 7.75E-137  |
| gene  | R         | P value     |
|-------|-----------|-------------|
| RND3  | 0.759434989 | 8.35E-81   |
| NKX3-1 | 0.377412248 | 8.42E-16   |
| IL1RN | 0.521179203 | 6.70E-31   |
| EGR1  | 0.65507106  | 2.53E-53   |
| SIK1  | 0.183678579 | 0.000142715|
| ZBTB21| 0.569493292 | 8.06E-38   |
| ACSM1 | -0.232334419| 1.32E-06   |
| C11orf96 | 0.540192663 | 1.72E-33   |
| FRMD4B| 0.570862829 | 4.94E-38   |
| PNRC1 | 0.554061155 | 1.74E-35   |
| RASGEF1B | 0.614365307 | 2.35E-45   |
| CRHBP | 0.838876617 | 1.57E-113  |
| FOS   | 0.649729233 | 3.30E-52   |
| EPHA2 | 0.64973961  | 3.28E-52   |
| MAP2K3| 0.442600682 | 9.12E-22   |
| AGTR1 | 0.362267216 | 1.35E-14   |
| IL1RAP| 0.487775864 | 9.98E-27   |
| DUSP5 | 0.376805324 | 9.43E-16   |
| FOSB  | 0.621342584 | 1.22E-46   |
| CYP26A1| 0.377013696 | 9.07E-16   |
| SOCS2 | 0.550341956 | 6.09E-35   |
| CDHR2 | 0.248323315 | 2.22E-07   |
| NR4A1 | 0.479459063 | 9.30E-26   |
| SCGB3A1| 0.333598862 | 1.76E-12   |
| PLIN2 | 0.428465956 | 2.32E-20   |
| CYP2C19| 0.295461269 | 5.47E-10   |
| APOF  | 0.659183616 | 3.37E-54   |
| JUN   | 0.536013307 | 6.60E-33   |
| gene   | R       | Pvalue   |
|--------|---------|----------|
| HAMP   | 0.539902643 | 1.89E-33 |
| MARCO  | 0.783004838  | 4.77E-89  |
| DCUN1D3 | 0.502215547  | 1.78E-28  |
| C8orf4 | 0.68592413    | 3.08E-60  |
| MBNL2  | 0.480446558   | 7.16E-26  |
| PTH1R  | 0.650663181   | 2.11E-52  |
| DNASE1L3 | 0.801160663  | 3.89E-96  |
| EHD3   | 0.547595603   | 1.52E-34  |
| VNN1   | 0.269170352   | 1.80E-08  |
| ECM1   | 0.791692936   | 2.37E-92  |
| TRIB1  | 0.64254245    | 9.64E-51  |
| INS-IGF2 | 0.667195699   | 6.09E-56  |
| DUSP6  | 0.582034823   | 8.27E-40  |
| IER2   | 0.451035694   | 1.23E-22  |
| ADAMTS13 | 0.8131215    | 3.20E-10  |
| LDLR   | 0.484532754   | 2.40E-26  |
| SLC38A2 | 0.603124373    | 2.35E-43  |
| TMEM150B | -0.30103768  | 2.49E-10  |
| DBH    | 0.649376975   | 3.90E-52  |
| ATF3   | 0.58838342    | 7.53E-41  |
| CLEC4M | 0.818714181   | 1.00E-103 |
| ADGRG7 | 0.440741125   | 1.41E-21  |
| RCAN1  | 0.691037223   | 1.82E-61  |
| CFP    | 0.793358869   | 5.29E-93  |
| CCL23  | 0.653902678   | 4.45E-53  |
| MAFF   | 0.48561677    | 1.79E-26  |
| PZP    | 0.534926631   | 9.33E-33  |
| COL5A3 | -0.225659085  | 2.68E-06  |
| gene        | R         | P-value     |
|------------|-----------|-------------|
| SERTAD1    | 0.57143375| 4.02E-38    |
| DTX1       | 0.34683543| 1.98E-13    |
| SERPINB8   | 0.50327664| 1.32E-28    |
| SERPINE1   | 0.566790797| 2.11E-37   |
| TMEM45B    | -0.274509652| 9.11E-09  |
| FAM46A     | 0.44957588| 1.74E-22    |
| ID1        | 0.449160176| 1.93E-22   |
| SOCS3      | 0.610388687| 1.22E-44   |
| KLF10      | 0.48518018| 2.02E-26    |
| PDE7B      | 0.29284786| 7.87E-10    |
| CLEC4G     | 0.822865538| 1.21E-105  |
| C21orf91   | 0.545000083| 3.59E-34   |
Table 4
167 hub genes in brown module.

| gene     | R       | Pvalue    |
|----------|---------|-----------|
| MFAP3L   | 0.708332134 | 7.98E-66 |
| CYP8B1   | 0.674262607 | 1.59E-57 |
| CYP39A1  | 0.672495233 | 3.99E-57 |
| GLYAT    | 0.72487592 | 2.66E-70 |
| ACSL1    | 0.756415028 | 8.11E-80 |
| ATAD3C   | 0.191242557 | 7.40E-05 |
| SLC41A2  | 0.573761362 | 1.73E-38 |
| MRAP2    | -0.331271354 | 2.56E-12 |
| CYP2C8   | 0.727118352 | 6.20E-71 |
| ACAA1    | 0.642144996 | 1.16E-50 |
| C1RL     | 0.649007129 | 4.65E-52 |
| FAM149A  | 0.576610021 | 6.14E-39 |
| IGFALS   | 0.541474507 | 1.14E-33 |
| MTHFD1   | 0.589985513 | 4.08E-41 |
| KDM8     | 0.610795219 | 1.03E-44 |
| PLIN1    | 0.270043627 | 1.61E-08 |
| AGL      | 0.689529744 | 4.21E-61 |
| KLKB1    | 0.688855384 | 6.12E-61 |
| PLPP3    | 0.637742101 | 8.74E-50 |
| GBP7     | 0.524169628 | 2.69E-31 |
| GRAMD1C  | 0.519924174 | 9.80E-31 |
| LARP1B   | 0.495813267 | 1.09E-27 |
| N4BP2L1  | 0.6292647 | 3.90E-48 |
| ACACB    | 0.576258741 | 6.98E-39 |
| ABCC9    | 0.629707137 | 3.21E-48 |
| PPP1R3B  | 0.484923418 | 2.16E-26 |
| FAM151A  | 0.195123376 | 5.23E-05 |
| gene   | R       | Pvalue    |
|--------|---------|-----------|
| GPR146 | 0.5479  | 1.34E-34  |
| TSLP   | 0.5471  | 1.80E-34  |
| HAAO   | 0.5726  | 2.68E-38  |
| C6     | 0.7634  | 4.12E-82  |
| TMEM56 | 0.6667  | 7.92E-56  |
| DMGDH  | 0.7921  | 1.55E-92  |
| ALDH2  | 0.6912  | 1.73E-61  |
| GSTZ1  | 0.6765  | 4.79E-58  |
| PANK1  | 0.6196  | 2.61E-46  |
| GPD1   | 0.6489  | 4.96E-52  |
| CYP2B6 | 0.5666  | 2.27E-37  |
| ACADS  | 0.5289  | 6.25E-32  |
| MFSD2A | 0.5703  | 5.94E-38  |
| AVPR1A | 0.4901  | 5.29E-27  |
| ESR1   | 0.6674  | 5.23E-56  |
| UGT2B7 | 0.6075  | 4.06E-44  |
| GNE    | 0.7653  | 8.77E-83  |
| ACADL  | 0.6549  | 2.68E-53  |
| DBT    | 0.6133  | 3.66E-45  |
| ARHGEF26 | 0.5259 | 1.58E-31  |
| GHR    | 0.7517  | 2.55E-78  |
| ACSM3  | 0.5877  | 9.69E-41  |
| RDH16  | 0.6732  | 2.75E-57  |
| CISH   | 0.3567  | 3.64E-14  |
| CYP7A1 | 0.2337  | 1.15E-06  |
| CD302  | 0.5489  | 9.84E-35  |
| TMEM82 | 0.4380  | 2.62E-21  |
| MANF   | -0.4789 | 1.08E-25  |
| gene   | R       | P_value   |
|--------|---------|-----------|
| HAO1   | 0.70515869 | 5.31E-65 |
| HSD17B13 | 0.674732572 | 1.24E-57 |
| UROC1  | 0.615895787 | 1.24E-45 |
| CYP3A43 | 0.437311457 | 3.12E-21 |
| ACSM5  | 0.756191632 | 9.58E-80 |
| SARDH  | 0.701126962 | 5.69E-64 |
| TUBE1  | 0.692055111 | 1.03E-61 |
| GNMT   | 0.38163345  | 3.78E-16 |
| SHMT1  | 0.52516227  | 1.98E-31 |
| MASP1  | 0.620794153 | 1.55E-46 |
| TDO2   | 0.515428545 | 3.78E-30 |
| THRSP  | 0.451785227 | 1.02E-22 |
| SLC38A4| 0.658171839 | 5.55E-54 |
| FOXO1  | 0.507174418 | 4.28E-29 |
| LIPG   | 0.493195725 | 2.25E-27 |
| SCP2   | 0.797158162 | 1.64E-94 |
| ACSL4  | -0.328219703 | 4.17E-12 |
| ANXA10 | 0.619637109 | 2.54E-46 |
| ACADM  | 0.667373423 | 5.56E-56 |
| ASCL1  | 0.209370974 | 1.38E-05 |
| C8B    | 0.626935715 | 1.09E-47 |
| IYD    | 0.600449914 | 6.84E-43 |
| AGXT2  | 0.528054348 | 8.10E-32 |
| CPT2   | 0.598014703 | 1.80E-42 |
| FOLH1  | 0.499651101 | 3.70E-28 |
| KCNN2  | 0.627560098 | 8.26E-48 |
| TPPP2  | 0.623478152 | 4.88E-47 |
| LPA    | 0.561611781 | 1.30E-36 |
| gene       | R       | P-value   |
|------------|---------|-----------|
| EPB41L4B   | 0.527126167 | 1.08E-31  |
| SPRYD4     | 0.597942673 | 1.85E-42  |
| MAT1A      | 0.692762044 | 6.89E-62  |
| NR3C2      | 0.48882415  | 7.50E-27  |
| GLS2       | 0.560205776 | 2.12E-36  |
| EFHD1      | 0.345722286 | 2.39E-13  |
| ADGRA3     | 0.55203128  | 3.45E-35  |
| CPED1      | 0.774267591 | 7.09E-86  |
| AKR1D1     | 0.675980833 | 6.44E-58  |
| ETFDH      | 0.762402652 | 8.65E-82  |
| HAO2       | 0.657529512 | 7.61E-54  |
| GCGR       | 0.527333101 | 1.01E-31  |
| ACADSB     | 0.596682504 | 3.03E-42  |
| SPTBN2     | 0.501882844 | 1.96E-28  |
| C8A        | 0.736763302 | 9.98E-74  |
| TCAP       | 0.152462107 | 0.001640854 |
| RETREG1    | 0.574323268 | 1.41E-38  |
| FAHD2A     | 0.550624592 | 5.54E-35  |
| FAM198A    | 0.524969202 | 2.10E-31  |
| STEAP3     | 0.632875634 | 7.85E-49  |
| RNF152     | 0.579096084 | 2.46E-39  |
| RNF125     | 0.641016158 | 1.95E-50  |
| GSTM1      | 0.384186389 | 2.32E-16  |
| PLG        | 0.733183057 | 1.12E-72  |
| CBR4       | 0.593346978 | 1.12E-41  |
| CYP4V2     | 0.698622668 | 2.43E-63  |
| SLC35D1    | 0.685722866 | 3.44E-60  |
| BCKDHB     | 0.687818559 | 1.09E-60  |
| gene     | R       | Pvalue  |
|----------|---------|---------|
| ADH4     | 0.691452331 | 1.44E-61 |
| PBLD     | 0.646347127 | 1.63E-51 |
| BHMT     | 0.615284859 | 1.60E-45 |
| SLC10A1  | 0.661759534 | 9.41E-55 |
| GIPC2    | 0.471811703 | 6.87E-25 |
| AQP8     | 0.108762184 | 0.025117783 |
| FNIP2    | 0.420614667 | 1.32E-19 |
| AL163636.2 | 0.553952254 | 1.80E-35 |
| CYP4A22  | 0.719920777 | 6.30E-69 |
| TMEM25   | 0.445881682 | 4.21E-22 |
| SLC22A1  | 0.714090299 | 2.40E-67 |
| SLC28A1  | 0.556632582 | 7.24E-36 |
| CYP4F2   | 0.695437305 | 1.51E-62 |
| MUT      | 0.631814537 | 1.26E-48 |
| SLC25A47 | 0.651159476 | 1.67E-52 |
| SRD5A1   | 0.569599329 | 7.76E-38 |
| ARID3C   | 0.56035738  | 2.01E-36 |
| BBOX1    | 0.507440711 | 3.96E-29 |
| CNDP1    | 0.695728435 | 1.28E-62 |
| INHBC    | 0.538899512 | 2.61E-33 |
| TTC36    | 0.706220896 | 2.82E-65 |
| ALDH6A1  | 0.766571262 | 3.38E-83 |
| SORL1    | 0.59075525  | 3.04E-41 |
| OXT      | 0.145041772 | 0.00275688 |
| BDH2     | 0.458952656 | 1.77E-23 |
| DNAJC25  | 0.650881637 | 1.90E-52 |
| NR1I2    | 0.723763852 | 5.44E-70 |
| NAT1     | 0.526659629 | 1.25E-31 |
| gene    | R       | Pvalue    |
|---------|---------|-----------|
| KLHL15  | 0.463894317 | 5.17E-24  |
| GCDH    | 0.553833852 | 1.88E-35  |
| CYP4A11 | 0.710255085 | 2.50E-66  |
| ADRA1A  | 0.762919911 | 5.80E-82  |
| GYS2    | 0.874170018 | 1.84E-134 |
| DIRAS3  | 0.58941734  | 5.07E-41  |
| SLC39A14| 0.438152534 | 2.57E-21  |
| F9      | 0.721709237 | 2.03E-69  |
| SRD5A2  | 0.542233738 | 8.87E-34  |
| ATP11C  | 0.562459079 | 9.67E-37  |
| ALDH8A1 | 0.697494997 | 4.66E-63  |
| ABAT    | 0.781938742 | 1.18E-88  |
| XDH     | 0.649046132 | 4.56E-52  |
| TMEM27  | 0.547045359 | 1.83E-34  |
| MPDZ    | 0.683794604 | 9.85E-60  |
| GLYATL1 | 0.878232527 | 2.84E-137 |
| CPEB3   | 0.7108366  | 1.76E-66  |
| AADAT   | 0.858905647 | 1.03E-124 |
| NDRG2   | 0.614351631 | 2.36E-45  |
| LCAT    | 0.639416196 | 4.07E-50  |
| TGFB3   | 0.367777945 | 5.01E-15  |
| ZFP1    | 0.672249892 | 4.53E-57  |
| ACAA2   | 0.622192623 | 8.50E-47  |
| EPHX2   | 0.71855342  | 1.49E-68  |
| FAM13A  | 0.508069599 | 3.30E-29  |
| GNAO1   | 0.556588803 | 7.35E-36  |
| SLC46A3 | 0.587857058 | 9.21E-41  |
| MOGAT2  | 0.747166675 | 7.00E-77  |
| gene     | R         | Pvalue   |
|----------|-----------|----------|
| MFAP3L   | 0.708332134 | 7.98E-66 |
| CYP8B1   | 0.674262607 | 1.59E-57 |
| CYP39A1  | 0.672495233 | 3.99E-57 |
| GLYAT    | 0.72487592  | 2.66E-70 |
| ACSL1    | 0.756415028 | 8.11E-80 |
| ATAD3C   | 0.191242557 | 7.40E-05 |
| SLC41A2  | 0.573761362 | 1.73E-38 |
| MRAP2    | -0.331271354 | 2.56E-12 |
| CYP2C8   | 0.727118352 | 6.20E-71 |
| ACAA1    | 0.642144996 | 1.16E-50 |
| C1RL     | 0.649007129 | 4.65E-52 |
| FAM149A  | 0.576610021 | 6.14E-39 |
| IGFALS   | 0.541474507 | 1.14E-33 |
| MTHFD1   | 0.589985513 | 4.08E-41 |
| KDM8     | 0.610795219 | 1.03E-44 |
| PLIN1    | 0.270043627 | 1.61E-08 |
| AGL      | 0.689529744 | 4.21E-61 |
| KLKB1    | 0.688855384 | 6.12E-61 |
| PLPP3    | 0.637742101 | 8.74E-50 |
| GBP7     | 0.524169628 | 2.69E-31 |
| GRAMD1C  | 0.519924174 | 9.80E-31 |
| LARP1B   | 0.495813267 | 1.09E-27 |
| N4BP2L1  | 0.6292647  | 3.90E-48 |
| ACACB    | 0.576258741 | 6.98E-39 |
| ABCC9    | 0.629707137 | 3.21E-48 |
| PPP1R3B  | 0.484923418 | 2.16E-26 |
| FAM151A  | 0.195123376 | 5.23E-05 |
| GPR146   | 0.547979178 | 1.34E-34 |
| gene   | R          | P.value     |
|--------|------------|-------------|
| TSLP   | 0.547086951 | 1.80E-34    |
| HAAO   | 0.572562573 | 2.68E-38    |
| C6     | 0.763363972 | 4.12E-82    |
| TMEM56 | 0.666678265 | 7.92E-56    |
| DMGDH  | 0.792166502 | 1.55E-92    |
| ALDH2  | 0.691127401 | 1.73E-61    |
| GSTZ1  | 0.676542082 | 4.79E-58    |

Table 5
Basic information about hsa_circ_0077210 via circbase database

| hsa_circ_0077210 | information          |
|------------------|----------------------|
| Position         | chr6:83933478–83938650 |
| Strand           | -                    |
| Host gene Symbol | ME1                  |
| bestTranscript   | NM_002395            |
| Length           | 423                  |
| Expression (Log \_ FC) | -2.641235   |
| Padjust          | 0.0000000478         |

**Supplemental Figure**
Figure S1 not available with this version

**Figures**
Figure 1

The screening of differentially expressed miRNAs and mRNAs.
Figure 2

The heatmap of differentially expressed miRNAs and mRNAs in TCGA-LIHC database
Figure 3

WGCNA analysis and identification of liver cancer tumorigenesis modules in miRNA expression profile.
Figure 4

WGCNA analysis and identification of liver cancer tumorigenesis modules in mRNA expression profile.
Figure 5

Survival analysis and pathway analysis of the miRNA in the most significant module.
Figure 6

Functional enrichment analysis of the co-expressed mRNAs in the key modules.
Figure 7

Screen of miRNA-mRNA interactions and target mRNAs.
Figure 8

Gene set enrichment analysis of target mRNAs.
Figure 9

Construction of CeRNA regulatory axis
Figure 10

Expression validation of CeRNA regulatory axis by qRT-PCR and HPA database

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.
• FigureS2.pdf
• FigureS3.pdf