FCN3 Is a Prognostic Biomarker Correlated with Immune Response in Liver Cancer

Zhongxiao Lu  
Xiamen University  https://orcid.org/0000-0001-8241-8643

Jian Wu  
Ningbo No.2 Hospital

Yi-ming Li  
Zhongshan Hospital,Xiamen University

Wen-xiang Chen  
Zhongshan Hospital,Xiamen University

Qiang-feng Yu  
the second hospital of LongYan

Jian-Yin Zhou (zhoujianyin2000@sina.com)  
Zhongshan Hospital,Xiamen university

Primary research

Keywords: FCN3, prognosis, immune response, liver cancer

DOI: https://doi.org/10.21203/rs.3.rs-90864/v1

License: Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Aim

Liver cancer is a common malignant tumor whose molecular pathogenesis remains unclear. This study attempts to identify key genes related to liver cancer by bioinformatics analysis and analyze their biological functions.

Methods

The gene expression data of the microarray were downloaded from the Gene Expression Omnibus (GEO) database. The differentially expressed genes (DEGs) were then identified by the R software package “limma” and were subjected to gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses using DAVID. The protein-protein interaction (PPI) network was constructed via String, and the results were visualized in Cytoscape. Modules and hub genes were identified using the MCODE plugin, while the expression of hub genes and its effects were analyzed by GEPIA2. Additionally, the co-expression of the hub gene was explored in String, while the GO results were visualized using the R software. Finally, the targets of the hub gene were predicted through an online website.

Results

In total, 43 differentially expressed genes were obtained. The GO analysis was mainly concentrated in the redox process and nuclear mitosis, while the KEGG pathway analysis was mainly enriched in retinol metabolism and the cell cycle. Moreover, four hub genes were identified in the PPI network, however, the Kaplan-Meier risk curve showed that only ECT2 and FCN3 affected the survival of liver cancer. ECT2 was found to be high expressed in liver cancer, carrying out signal transduction and targeting hsa-miR-27a-3p. FCN3 was observed to be lowly expressed in liver cancer and related to the immune response, targeting hsa-miR132-5p.

Conclusion

The obtained findings suggest that two genes are significantly related to the prognosis of liver cancer, and the analysis of their biological function provided novel insight into the pathogenesis of liver cancer. Furthermore, FCN3 may serve as a promising biomarker for patients with liver cancer.

1. Introduction

Liver cancer is the most common primary malignant tumor of the liver with more than 800,000 newly diagnosed cases worldwide each year[1]. It has one of the highest incidence and mortality rates of cancer in the world[2], and its morbidity and mortality rates are demonstrating upward trends in many countries[3]. It is the fourth leading cause of cancer-related deaths among humans, heavily burdening both patients and society. The occurrence of liver cancer is related to a variety of risk factors, in conjunction with common causes such as liver cirrhosis, viral infection, smoking, alcoholism, metabolic
diseases, nonalcoholic fatty liver, obesity, heredity, and gene mutation[4-8]. Other studies have shown that adenovirus infection[9] may serve as a new cause of liver cancer in a small number of individuals. Although a variety of treatments for liver cancer exist, including surgical resection, radiofrequency ablation, sorafenib targeted therapy, chemotherapy, liver transplantation [10-14] and emerging immunotherapy[15, 16], due to the high recurrence rate, the 3-year survival rate and 5-year survival rate are still very low[17]. Hence, identifying reliable and effective biomarkers to evaluate the diagnosis, treatment and prognosis of patients with liver cancer is urgently needed.

Due to the development of bioinformatics, GEO has been widely used to identify DEGs, after which identifying biomarkers from DEGs may be performed. The differentially expressed genes between cancer and paracancerous tissues were identified using gene expression microarrays, while certain tumor-related biomarkers were discovered from differentially expressed genes through a series of methods, providing a basis for a follow-up study of the pathogenesis of tumor through cell experimentations. An increasing number of studies have used this method in order to ascertain the biomarkers of tumors[18-20], For example, ZihaoHe et al. found that the overexpression of ABCC4 or low expression of SLPI exhibited a poor prognosis in patients with prostate cancer through the GSE103512 dataset in the GEO database[21]. However, at present, precise research regarding the bioinformatics of liver cancer is not uncommon, however, no unified conclusion exists. Accordingly, the detection of reliable biomarkers in liver cancer is particularly important for the early diagnosis and treatment of liver cancer.

Therefore, in this study, the gene expression profiles of 158 pairs of liver cancer and normal liver tissues were compared in order to find the differentially expressed genes (DEGs). Subsequently, function and pathway enrichment analyses were carried out to identify their main functions and enriched signal pathways of the differentially expressed genes in liver cancer to determine the key nodes involved in the maintenance of these pathways. In addition, these genes were further analyzed by the prognostic model. Finally, the expression of the genes and targeted miRNA were analyzed in order to offer new insight for clinicians in the diagnosis and treatment of liver cancer.

2. Materials And Methods

2.1 Data acquisition

Three microarray datasets of liver cancer, GSE64041 (platform: GPL6244), GSE54236 (platform: GPL6480) and GSE60502 (platform: GPL96) were downloaded from the GEO (https://www.ncbi.nlm.nih.gov/geo/) database. The samples were required to meet the following inclusion criteria[1]: 1) Data contained only mRNA that were expressed 2) Each tumor sample had a corresponding control. After excluding healthy donors and unpaired samples, 120 samples, 160 samples and 36 samples were obtained from the three microarray datasets, respectively.

2.2 Data processing and identification of differentially expressed genes
GSE64041 and GSE54236 were used for the primary identification of DEGs, while GSE60502 was used for the verification of DEGs. First, the data quality of the three microarray datasets was evaluated using the affyPLM package of the R software and the samples were preprocessed using the RMA Method for normalization. Afterward, the “limma” package of the R software was used to identify the DEGs[22] between cancer and normal samples in GSE64041 and GSE54236. R software was then used to draw heat maps and volcano maps, while GEO2R was used to identify the differentially expressed genes in GSE60502. The criteria for identifying the DEGs were as follows: absolute value of log2FC > 1 and P < 0.05. Finally, the Venn diagram of differentially expressed genes shared by the three microarray datasets was drawn by the software funrich.

2.3 GO and KEGG analysis of differentially expressed genes

The differentially expressed genes identified by GSE64041 and GSE54236 were analyzed through GO and KEGG using the online database DAVID6.8 (https://david.ncifcrf.gov/). GO analysis includes three items: biological process, cell composition and molecular function. KEGG is used to explain the biological pathways of gene enrichment[23]. P < 0.05 was taken as the cut-off standard, and the results were visualized by the R software.

2.4 Construction of protein-protein interaction network and module analysis

The DEGs shared by the three microarrays were inputted into String (http://string-db.org/), an online website, which was also used to build the PPI network[24]. The identifying criteria were set as the interaction score > 0.4. The constructed PPI network was then visualized by the Cytoscape software, and the hub gene was identified using the plug-in MCODE in Cytoscape.

2.5 Survival analysis

GEPIA2 (http://gepia2.cancer-pku.cn/) was used to analyze whether the expression of the hub gene was related to the survival of liver cancer patients. GEPIA2, like GEPIA, is sampled from The Cancer Genome Atlas(TCGA)[25]. According to the median value of gene expression, the patients were divided into two groups. One group had high expression while the other had low expression. The overall survival (OS) of patients was analyzed using the Kaplan-Meier method. P < 0.05 was considered to be statistically significant.

2.6 Expression of the hub genes

GEPIA2 was utilized to analyze the expression of two hub genes (ECT2 and FCN3), which influenced the prognosis. After the target gene was imported into GEPIA2, its expression in common tumors was analyzed(Figure.8A,Figure.8B), followed by its expression in liver cancer.

2.7 Co-expression and functional analysis of the hub genes
In order to determine the co-expression genes of the hub genes, the selected hub genes (ECT2 and FCN3) were analyzed by String, after which the co-expression network was visualized by Cytoscape. Next, ECT2 and FCN3, along with their co-expression genes, were analyzed by GO using R-packet clusterProfiler, and the analysis results were visualized by R-packet Goplot. Finally, the functions of ECT2 and FCN3 were analyzed by the online database DAVID6.8. The results were then recorded in a table.

2.8 Prediction of targeted miRNA

Starbase (http://starbase.sysu.edu.cn/), was used to predict the targeted miRNA of ECT2 and FCN3, and the correlation between the target gene and miRNA was analyzed. StarBase is an online network program used to determine the targeted miRNA of the target gene by collecting published articles. Due to the large number of targeted miRNA for ECT2, further identification and verification was carried out in miRmap (https://mirmap.ezlab.org/) and miRanda (http://www.miranda.org/).

3. Results

3.1 General characteristics of three microarray datasets

Three microarray datasets were downloaded from the GEO database. Their characteristics are shown in Table 1.

Table 1. The basic information of three microarray datasets

| GEO datasets | Platform | Normal Number | Tumor Number | Type |
|--------------|----------|---------------|--------------|------|
| GSE64041     | GPL6244  | 60            | 60           | Paired |
| GSE54236     | GPL6480  | 80            | 80           | Paired |
| GSE60502     | GPL96    | 18            | 18           | Paired |

3.2 Identifying differentially expressed genes

In total, 317 and 342 DEGs were obtained using the R software package DESeq from the GSE64041 and GSE54236 datasets, respectively. The number of DEGs upregulated in GSE64041 and GSE54236 was 87 and 146, respectively. Additionally, the number of DEGs downregulated was 230 and 196, respectively. The heat and volcano maps in Figure 2 demonstrate the differentially expressed genes.

3.3 GO and KEGG Pathway analysis of differentially expressed genes

The GO analysis demonstrated that for GSE64041, among the types of biological processes, differentially expressed genes were mainly enriched in the oxidation-reduction process, mitotic nuclear division, tryptophan catabolic process to kynurenine, cellular response to tumor necrosis factor and tryptophan...
catabolic process. The cell composition analysis showed that most of the differentially expressed genes mainly played a role in the extracellular region. In terms of molecular function, these differentially expressed genes exhibited consistency in monooxygenase activity, iron ion binding, heme binding, oxidoreductase activity, acting on paired donors and oxygen binding (Figure.3A). In regard to GSE54236, in the enrichment of biological processes, the main functions of the differentially expressed genes were found to be mitotic nuclear division, cell division, sister chromatid cohesion, chromosome segregation, G2/M transition of mitotic cell cycle, and so forth. The cell composition analysis showed that most differentially expressed genes were mainly located on chromosomes. According to the results of the molecular functional analysis, differentially expressed genes were found to be mainly related to microtubule binding, ATP-dependent microtubule motor activity, microtubule motor activity, protein kinase binding and protein binding (Figure.3B). KEGG analysis showed that the differentially expressed genes of GSE64041 were mainly involved in cell cycle, oocyte meiosis, metabolic pathways, cell cycle and PI3K-Akt signaling pathway(Figure.3C). However, the DEGs of GSE54236 were observed to be mainly related to cell cycle, oocyte meiosis, progesterone-mediated oocyte maturation, p53 signaling pathway and histidine metabolism (Figure.3D).

3.4 Identification of DEGs

After summing the first 250 differentially expressed genes in GSE64041 and GSE54236, as well as all 216 differentially expressed genes in GSE60502, 43 differentially expressed genes were obtained in the three microarray datasets (Figure.4).

3.5 Construction of PPI network with DEGs

After the PPI network of 43 common DEGs was constructed using String, 33 differentially expressed genes were located on the PPI network nodes (Figure.5A). The PPI network visualization results can be seen in Fig.5B.

3.6 Functional modules and hub genes in the PPI

In the PPI network, two functional modules may be noted (Fig. 6A and Fig. 6B). According to the confidence > 0.4 and node > 10, four hub genes were identified: Epithelial Cell Transforming Sequence2 (ECT2), Stabilin-2 (STAB2), ficolin 2 (FCN2) and ficolin 3 (FCN3) (Fig. 6C).

3.7 Survival analysis

In order to analyze the relationship between the identified hub gene and overall survival rate of liver cancer, the survival curve of the four hub genes was analyzed using GEPIA2. According to the median of gene expression, Patients were divided into a high expression group and low expression group. The expression of ECT2 (P=0.00091) and FCN3 (P=0.033) was correlated with OS in liver cancer patients. A higher expression of ECT2 in liver cancer patients signified a shorter OS, whereas a higher expression of FCN3 meant a longer OS (Figure.7)
3.8 Expression of hub gene

The expression of the two hub genes was further confirmed by GEPIA2. In most tumors, ECT2 showed high expression (Figure.8A), while FCN3 showed low expression (Figure.8B). In liver cancer (LIHC), ECT2 also demonstrated high expression (Figure.8C), while FCN3 demonstrated low expression, as in most other tumors (Figure.8D).

3.9 Co-expression and Biological process of the hub genes

The co-expression genes of ECT2 obtained from String were PLK1, CDK1, RHOB, RHO C, RACGAP, RHOA, KIF23, CDC42, ASPM, and CENPE. The co-expression genes of FCN3 were COLEC10, COLEC11, FCN1, FCN2, C4B, C4A, MASP2, C2, MBL2, and MASP1 (Figure.9A and Figure.9B). The results of the GO analysis showed that ECT2 and its related co-expression genes were mainly associated with cell morphogenesis in the biological process (Figure.9C). However, FCN3 and its co-expression genes were found to be significantly enriched in complement activation (Figure.9F). In addition, the cell composition analysis illustrated that ECT2 and its co-expression genes were mainly located in the nucleus (Figure.9D), while FCN3 and its co-expression genes were distributed in the extracellular region (Figure.9G). According to the results of the molecular functional analysis, ECT2 and co-expression genes were mainly related to signal transducer activity (Figure.9E), while FCN3 and its co-expression genes were mainly related to antigen binding (Figure.9H). The enrichment results of ECT2 and FCN3 in DAVID were also found to be consistent with those of the R software (Table.2).

Table.2 The main biological processes involved in the hub genes

| Gene   | Term                        | Involved in                  |
|--------|-----------------------------|------------------------------|
| ECT2   | GO:0000902                  | cell morphogenesis           |
|        | GO:0005634                  | nucleus                      |
|        | GO:0004871                  | signal transducer activity   |
| FCN3   | GO:0001867                  | complement activation        |
|        | GO:0005576                  | extracellular region         |
|        | GO:0003823                  | antigen binding              |

3.10 Targeting miRNA of the hub genes

In order to understand the potential targeting miRNA of the hub genes in liver cancer, Starbase, miRmap and miRanda were used to predict the targeted miRNA of ECT2 and FCN3. Accordingly, 29 genes were collected as target genes of ECT2 in different tumor types. Hsa-miR-27a-3p and hsa-miR-27b-3p were
selected as target miRNA as they were the most demonstrated in the corresponding experiments. Here, two targeted miRNA of FCN3 were collected: hsa-miR-217 and hsa-miR-132-5p (Table.3).

Table.3 Targeting miRNA of hub gene

| Gene  | miRNA            |
|-------|------------------|
| ECT2  | hsa-miR-27a-3p   |
|       | hsa-miR-27b-3p   |
| FCN3  | hsa-miR-217      |
|       | hsa-miR-132-5p   |

4. Discussion

The occurrence and development of tumors is a complex process involving multiple genes and metabolic pathways. The self-renewal ability of cancer stem cells is considered to be the main reason promoting cancer progression and resistance to drug therapy[26]. Numerous patients with hepatitis B exist in China, where liver cancer is a common malignant tumor. Due to discrete early clinical symptoms, lack of sensitive and specific markers, and limited diagnostic methods patients are often treated during the middle and late stages of disease. The survival rate has not improved much in recent years [27, 28]. Therefore, determining the key molecules causing liver cancer, exploring its internal mechanisms and finding potential therapeutic targets are future research directions. The rise of high-throughput sequencing technology has made the search for tumor markers very convenient. This technique can measure the expression level of thousands of genes simultaneously, which is a powerful tool to study the gene expression profiles between cancer and normal samples. Furthermore, this method provides a theoretical basis for our experimental follow-up cell study[29].

Since research pertaining to the key molecules and pathogenesis of liver cancer is not very clear, three microarray datasets were downloaded from GEO, which encompassed 158 pairs of samples. GSE64041 and GSE54236 were used to identify the differentially expressed genes. After they were identified, the function and pathways of these DEGs were studied.

Accordingly, 317 DEGs were identified in GSE64041, where 87 differentially expressed genes were found to be upregulated while 230 differentially expressed genes were found to be downregulated. The GO analysis showed that the differentially expressed genes were mainly enriched in the oxidation-reduction process, mitotic nuclear division, tryptophan catabolic process, kynurenine, cellular response to tumor necrosis factor, tryptophan catabolic process, monooxygenase activity, iron ion binding, heme binding, oxidoreductase activity, acting on paired donors and oxygen binding. In the KEGG pathway analysis, these DEGs were mainly observed to be related to retinol metabolism, oocyte meiosis, metabolic
pathways, cell cycle, PI3K-and Akt signaling pathway. However, 342 DEGs were identified in GSE54236, in which the number of differentially expressed genes that were upregulated and downregulated were 146 and 196, respectively. The GO functional enrichment analysis demonstrated that the differentially expressed genes were mainly enriched in mitotic nuclear division, cell division, sister chromatid cohesion, chromosome segregation, G2/M transition of mitotic cell cycle, microtubule binding, ATP-dependent microtubule motor activity, microtubule motor activity, protein kinase binding, and protein binding. According to the KEGG analysis, the differentially expressed genes were mainly involved in the cell cycle, oocyte meiosis, progesterone-mediated oocyte maturation, p53 signaling pathway, and histidine metabolism. Tumors are the result of unregulated cell division and proliferation, a process that consumes much energy. Many different types of tumors, including breast cancer, kidney cancer, lung cancer, prostate cancer and colorectal cancer, have differences in metabolism[30-33]. The process of glucose metabolism in tumor cells is significantly different from that in normal cells[34, 35]. Studies have pointed out that in tumor cells, the mitochondria that produce energy are damaged, hence, most tumor cells use glucose and amino acids as substrates. Moreover, under aerobic conditions, energy is obtained only through anaerobic glycolysis outside the mitochondria, termed the "Warburg" effect[36, 37]. This type of metabolism is inefficient and consumes more energy while producing the same amount of ATP as aerobic oxidation, resulting in loss of weight in patients with advanced cancers. Monoxygenase is also called liver microsomal enzyme in the liver. The main component of monoxygenase is cytochrome P450 (CYPs), which is mainly involved in detoxification and drug metabolism (including retinol metabolism). Studies have pointed out that CYP450 family genes are significantly associated with liver disease and liver cancer. For example, the downregulation of CYP2A6 and CYP2C8 is related to the overall survival and recurrence rates of liver cancer[38]. Additionally, the high expression of CYP4A11 is associated with a better prognosis in patients with liver cancer[39], and CYP activity is affected in varying degrees in patients with liver fibrosis or cirrhosis. The PI3K-Akt pathway is a common pathway in cells[40], which is considered to play a role in accelerating the cell cycle, promoting cell proliferation and inhibiting cell apoptosis. Currently, certain targeted therapeutic drugs designed for this pathway have made some progress[41, 42], and inhibitors targeting PI3K and AKT have also entered clinical trials [43]. Chromosome separation and sister chromatid binding are important processes in cell division. Chih-JuiChang et al. found that DNA topoisomerase (TOPO) is essential for the later stages of sister chromatid separation[44]. TP53 encodes the p53 protein, which is an important regulatory factor of the cell cycle, that induces cell cycle arrest and regulates cell apoptosis[45]. The inactivation of the p53 protein is a late event of liver cancer, increasing the malignant degree of liver cancer and contributing to resistance to treatment[46]. Another study suggested that the TP53 pathway is related to immunity. In this regard, TP53 mutation can promote the expression of PD-L1 and increase the infiltration of T lymphocytes in lung adenocarcinoma[47].

In order to determine the final hub gene, the third microarray dataset was used to overlap the differentially expressed genes of the three microarray datasets, resulting in 43 hub genes. By establishing the PPI network and using Cytoscape plugins, four hub genes were finally identified, of which two were found to be related to prognosis: ECT2 and FCN3.
ECT2 is epithelial transformation sequence 2[48] and a guanosine nucleotide exchange factor (GEFs), which catalyzes the transformation between GDT and GTP, activating Rho enzyme[49] and regulating cell division[50]. Many studies have found that ECT2 is abnormally expressed in many tumors. For example, Zhang et al found that ECT2 is overexpressed in pancreatic cancer and is related to methylation [51]. Moreover, Sano et al. confirmed the high expression of ECT2 in gliomas, which predicted poor prognosis [52]. Xu et al also confirmed that ECT2 and miR-223 form an axis of action and regulate osteosarcoma development [53]. An increasing number of studies have found that ECT2 regulates tumor progression through a variety of ways, plays a carcinogenic role in mistakenly activating Rho [54, 55] overactivates the ras/mapk pathway, which leads to tumor formation[56] and promotes tumor cell invasion by regulating the EMT process [57]. The mechanism of ECT2 in liver cancer has also been studied, which is consistent with the results of the present study in regard to ECT2. Chen et al believed that the expression of ECT2 is upregulated in liver cancer, and ECT2 can promote the expression of the related gene RACGAP1, which mediates the activation of Rho enzyme, leading to the early recurrence of liver cancer [58].

FCN3 is a member of the FCN gene family, which varies greatly between races [59]. The results of this study demonstrated that the expression of FCN3 was found to be decreased in liver cancer, where FCN3 was mainly observed to be involved in complement activation, consistent with the results of other studies. Accordingly, it is suggested that FCN3 is highly expressed in normal liver tissues but stably low in liver cancer. Compared to FCN1 and FCN2, FCN3 has a higher ability with respect to complement activation. [60, 61]. In addition, the expression of FCN3 was found to be closely related to the disease. For example, Chen et al. found that the decrease in serum FCN3 was associated with insulin resistance, while lower serum FCN3 predicted the development of type 2 diabetes [62]. Zheng et al. discovered that the expression of FCN3 increased in the vitreous effusion of patients with proliferative diabetic retinopathy, hence, FCN3 may serve as a new therapeutic target for the treatment of proliferative diabetic retinopathy [63]. Szala et al. found that the expression of FCN3 in patients with ovarian cancer was significantly lower than that in benign ovarian tumors and normal ovarian tissues [64]. Shi et al. put forward that the expression of FCN3 was low in lung squamous cell carcinoma [65]. The mechanism of FCN3 in liver cancer has yet to be reported, which warrants further elucidation.

At present, AFP is still used as a diagnostic marker for liver cancer. Due to the lack of good sensitivity and specificity, missed diagnoses and misdiagnosis may often occur. Although liver cancer markers have repeatedly emerged over time, no unified conclusion currently exists[66-69]. In this study, differentially expressed genes were found from a large dataset (316 samples in total). In order to improve accuracy, the identified genes were verified in TCGA, in which a difference in their expression was observed (Figure.8). Afterward, their function, prognosis and targeted miRNA were analyzed. Overall, this study provided novel insights in understanding the pathogenesis of liver cancer as well as the search for tumor markers. The mechanism of FCN3 in diabetes, ovarian cancer and lung squamous cell carcinoma has previously been studied, but its role in liver cancer is not clear. In the future, we intend to verify the expression of FCN3 in tissues, identify its downstream target genes, and explore the effects of its expression changes on the proliferation and invasion of liver cancer.
5. Conclusions

ECT2 and FCN3 are related to patient survival in liver cancer. The expression of ECT2 is upregulated in most tumors (including liver cancer), while FCN3 is downregulated. ECT2 is mainly involved in signal transduction, whereas FCN3 is involved in the immune response. The targeting miRNA of ECT2 is hsamiR-27a-3p, and the targeting miRNA of FCN3 is hsa-miR-132-5p. Since the mechanism of action of ECT2 in liver cancer has been reported, FCN3 has added research value in liver cancer.

Abbreviations

GEO: Gene Expression Omnibus; DEGs: differentially expressed genes; GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein-protein interaction; TCGA: The Cancer Genome Atlas;

Declarations

Author Contributions

Zhong-xiao Lu designed this study and wrote this paper, Jian Wu downloaded data, Yi-ming Li downloaded references, Wen-xiang Chen checked references, Qiang-feng Yu and Jian-yin Zhou reviewed the paper. Finally, all authors approved this version.

Author details

1Department of Hepatobiliary and Pancreatic Surgery, Zhongshan Hospital, Xiamen University, Xiamen, 361004, China. 2Ningbo No.2 Hospital, Ningbo, 315010, Zhejiang, China. 3Department of Hepatobiliary Surgery, the Second Hospital of Longyan, Fujian, 364000, China.

Acknowledgements

Not applicable.

Competing interests

The authors declare no benefits in any form have been received.

Availability of data and materials

All the data of this study can be obtained by contacting corresponding authors.

Consent for publication

Not applicable.

Ethics approval and consent to participate
All the data in this study are from open databases, and the relevant policies are followed in the process of downloading and using the data. Therefore, there is no need for the Ethics Committee to approve this study.

**Funding**

This research received funding from the Department of Science and Technology of Fujian Province (2019J01551)

**References**

1. Bray, F., et al., *Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries*. CA Cancer J Clin, 2018. **68**(6): p. 394-424.

2. Ferlay, J., et al., *Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012*. Int J Cancer, 2015. **136**(5): p. E359-86.

3. Torre, L.A., et al., *Global cancer statistics, 2012*. CA: A Cancer Journal for Clinicians, 2015. **65**(2): p. 87-108.

4. Massarweh, N.N. and H.B. El-Serag, *Epidemiology of Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma*. Cancer Control, 2017. **24**(3).

5. Ye, X., et al., *Genetic variants of ALDH2-rs671 and CYP2E1-rs2031920 contributed to risk of hepatocellular carcinoma susceptibility in a Chinese population*. Cancer Manag Res, 2018. **10**: p. 1037-1050.

6. Wang, J., et al., *Insight into the molecular mechanism of LINC00152/miR-215/CDK13 axis in hepatocellular carcinoma progression*. J Cell Biochem, 2019. **120**(11): p. 18816-18825.

7. Younossi, Z.M., et al., *Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009*. Hepatology, 2015. **62**(6): p. 1723-1730.

8. Huang, J.T., et al., *Next generation digital PCR measurement of hepatitis B virus copy number in formalin-fixed paraffin-embedded hepatocellular carcinoma tissue*. Clin Chem, 2015. **61**(1): p. 290-6.

9. Nault, J.C., et al., *Recurrent AAV2-related insertional mutagenesis in human hepatocellular carcinomas*. Nat Genet, 2015. **47**(10): p. 1187-93.

10. Xia, H., et al., *EGFR-PI3K-PDK1 pathway regulates YAP signaling in hepatocellular carcinoma: the mechanism and its implications in targeted therapy*. Cell Death & Disease, 2018. **9**(3).

11. Liao, Y., et al., *Sorafenib therapy following resection prolongs disease-free survival in patients with advanced hepatocellular carcinoma at a high risk of recurrence*. Oncol Lett, 2017. **13**(2): p. 984-992.

12. Cao, N., et al., *Application of curved ablation in liver cancer with special morphology or location: Report of two cases*. World Journal of Clinical Cases, 2020. **8**(9): p. 1713-1720.

13. Lee, J.H., et al., *ELK3 promotes the migration and invasion of liver cancer stem cells by targeting HIF-1 alpha*. Oncology Reports, 2017. **37**(2): p. 813-822.
14. Llovet, J.M., et al., Molecular therapies and precision medicine for hepatocellular carcinoma. Nat Rev Clin Oncol, 2018. 15(10): p. 599-616.

15. Akateh, C., et al., Neoadjuvant and adjuvant treatment strategies for hepatocellular carcinoma. World Journal of Gastroenterology, 2019. 25(28): p. 3704-3721.

16. Wang, H., et al., Effect of HK2, PKM2 and LDHA on Cetuximab efficacy in metastatic colorectal cancer. Oncology Letters, 2018.

17. Heimbach, J.K., et al., AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology, 2018. 67(1): p. 358-380.

18. Li, H.M., et al., Identification and interaction analysis of key genes and microRNAs in atopic dermatitis by bioinformatics analysis. Clin Exp Dermatol, 2019. 44(3): p. 257-264.

19. Liu, J., et al., Aberrantly methylated-differentially expressed genes and pathways in colorectal cancer. Cancer Cell Int, 2017. 17: p. 75.

20. Huang, Z., Q. Yang, and Z. Huang, Identification of Critical Genes and Five Prognostic Biomarkers Associated with Colorectal Cancer. Med Sci Monit, 2018. 24: p. 4625-4633.

21. He, Z., X. Duan, and G. Zeng, Identification of potential biomarkers and pivotal biological pathways for prostate cancer using bioinformatics analysis methods. PeerJ, 2019. 7: p. e7872.

22. Ritchie, M.E., et al., limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res, 2015. 43(7): p. e47.

23. Kanehisa, M., et al., KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res, 2016. 44(D1): p. D457-62.

24. Szklarczyk, D., et al., STRING v10: protein–protein interaction networks, integrated over the tree of life. Nucleic Acids Research, 2015. 43(D1): p. D447-D452.

25. Tang, Z., et al., GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Research, 2017. 45(W1): p. W98-W102.

26. Lee, I.C., S. Fadera, and H.L. Liu, Strategy of differentiation therapy: effect of dual-frequency ultrasound on the induction of liver cancer stem-like cells on a HA-based multilayer film system. J Mater Chem B, 2019. 7(35): p. 5401-5411.

27. Zhong, W., et al., Cholangiocarcinomaassociated genes identified by integrative analysis of gene expression data. Mol Med Rep, 2018. 17(4): p. 5744-5753.

28. Chen, D., et al., Screening differential circular RNA expression profiles reveal that hsa_circ_0128298 is a biomarker in the diagnosis and prognosis of hepatocellular carcinoma. Cancer Manag Res, 2018. 10: p. 1275-1283.

29. Li, X.N., et al., RNA sequencing reveals the expression profiles of circRNA and indicates that circDDX17 acts as a tumor suppressor in colorectal cancer. J Exp Clin Cancer Res, 2018. 37(1): p. 325.

30. Asiago, V.M., et al., Early Detection of Recurrent Breast Cancer Using Metabolite Profiling. Cancer Research, 2010. 70(21): p. 8309-8318.
31. Lin, L., et al., Direct infusion mass spectrometry or liquid chromatography mass spectrometry for human metabonomics? A serum metabonomic study of kidney cancer. Analyst, 2010. 135(11): p. 2970-8.

32. Lokhov, P.G., et al., Metabolic fingerprinting of blood plasma from patients with prostate cancer. Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry, 2010. 4(1): p. 37-41.

33. Rocha, C.M., et al., Metabolic Signatures of Lung Cancer in Biofluids: NMR-Based Metabonomics of Blood Plasma. Journal of Proteome Research, 2011. 10(9): p. 4314-4324.

34. DeWaal, D., et al., Hexokinase-2 depletion inhibits glycolysis and induces oxidative phosphorylation in hepatocellular carcinoma and sensitizes to metformin. Nature Communications, 2018. 9(1).

35. Mao, X., et al., Capsaicin inhibits glycolysis in esophageal squamous cell carcinoma by regulating hexokinase2 expression. Mol Med Rep, 2018. 17(4): p. 6116-6121.

36. Massari, F., et al., Metabolic phenotype of bladder cancer. Cancer Treat Rev, 2016. 45: p. 46-57.

37. Hanahan, D. and Robert A. Weinberg, Hallmarks of Cancer: The Next Generation. Cell, 2011. 144(5): p. 646-674.

38. Ren, X., et al., Downregulation of CYP2A6 and CYP2C8 in Tumor Tissues Is Linked to Worse Overall Survival and Recurrence-Free Survival from Hepatocellular Carcinoma. BioMed Research International, 2018. 2018: p. 1-9.

39. Eun, H.S., et al., Cytochrome P450 4A11 expression in tumor cells: A favorable prognostic factor for hepatocellular carcinoma patients. J Gastroenterol Hepatol, 2019. 34(1): p. 224-233.

40. Sohal, D.P., et al., Molecular characteristics of biliary tract cancer. Crit Rev Oncol Hematol, 2016. 107: p. 111-118.

41. Kudo, M., Signaling Pathway and Molecular-Targeted Therapy for Hepatocellular Carcinoma. Digestive Diseases, 2011. 29(3): p. 289-302.

42. Xia, H., et al., EGFR-PI3K-PDK1 pathway regulates YAP signaling in hepatocellular carcinoma: the mechanism and its implications in targeted therapy. Cell Death & Disease, 2018. 9.

43. Garlich, J.R., et al., A Vascular Targeted Pan Phosphoinositide 3-Kinase Inhibitor Prodrug, SF1126, with Antitumor and Antiangiogenic Activity. Cancer Research, 2008. 68(1): p. 206-215.

44. Chang, C.J., et al., RNAi analysis reveals an unexpected role for topoisomerase II in chromosome arm congression to a metaphase plate. J Cell Sci, 2003. 116(Pt 23): p. 4715-26.

45. M., et al., p53 Regulates the Minimal Promoter of the Human Topoisomerase II Gene. 1996.

46. Lim, I.K., Erratum to “Spectrum of molecular changes during hepatocarcinogenesis induced by DEN and other chemicals in Fischer 344 male rats” [Mechanisms of Ageing and Development 123 (2002) 1665–1680]. Mechanisms of Ageing and Development, 2003. 124(5): p. 697-708.

47. Dong, Z.-Y., et al., Potential Predictive Value of TP53 and KRAS Mutation Status for Response to PD-1 Blockade Immunotherapy in Lung Adenocarcinoma. Clinical Cancer Research, 2017. 23(12): p. 3012-3024.
48. Chen, J., et al., *ECT2 regulates the Rho/ERK signalling axis to promote early recurrence in human hepatocellular carcinoma*. Journal of Hepatology, 2015. 62(6): p. 1287-1295.

49. Rossman, K.L., C.J. Der, and J. Sondek, *GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors*. Nat Rev Mol Cell Biol, 2005. 6(2): p. 167-80.

50. Kimura, K., et al., *Accumulation of GTP-bound RhoA during cytokinesis and a critical role of ECT2 in this accumulation*. J Biol Chem, 2000. 275(23): p. 17233-6.

51. Zhang, M.-L., et al., *Correlation between ECT2 gene expression and methylation change of ECT2 promoter region in pancreatic cancer*. Hepatobiliary & Pancreatic Diseases International, 2008. 7(5): p. 533-538.

52. Sano, M., et al., *Expression level of ECT2 proto-oncogene correlates with prognosis in glioma patients*. Oncology Reports, 2006. 16(5): p. 1093-1098.

53. Xu, J., et al., *miR-223/Ect2/p21 signaling regulates osteosarcoma cell cycle progression and proliferation*. Biomedicine & Pharmacotherapy, 2013. 67(5): p. 381-386.

54. Saito, S., et al., *Deregulation and mislocalization of the cytokinesis regulator ECT2 activate the Rho signaling pathways leading to malignant transformation*. J Biol Chem, 2004. 279(8): p. 7169-79.

55. Huff, L.P., et al., *The Role of Ect2 Nuclear RhoGEF Activity in Ovarian Cancer Cell Transformation*. Genes Cancer, 2013. 4(11-12): p. 460-75.

56. Canevascini, S., et al., *The Caenorhabditis elegans homologue of the proto-oncogene ect-2 positively regulates RAS signalling during vulval development*. EMBO Rep, 2005. 6(12): p. 1169-75.

57. Chen, Z., J. Liu, and Y. Zhang, *Role of Epithelial Cell Transforming Sequence 2 (ECT2) in Predicting Prognosis of Osteosarcoma*. Medical Science Monitor, 2017. 23: p. 3861-3868.

58. Chen, J., et al., *ECT2 regulates the Rho/ERK signalling axis to promote early recurrence in human hepatocellular carcinoma*. J Hepatol, 2015. 62(6): p. 1287-95.

59. Hummelshoj, T., et al., *Functional SNPs in the human ficolin (FCN) genes reveal distinct geographical patterns*. Mol Immunol, 2008. 45(9): p. 2508-20.

60. Hummelshoj, T., et al., *Comparative study of the human ficolins reveals unique features of Ficolin-3 (Hakata antigen)*. Mol Immunol, 2008. 45(6): p. 1623-32.

61. Luo, J.H., et al., *Transcriptomic and genomic analysis of human hepatocellular carcinomas and hepatoblastomas*. Hepatology, 2006. 44(4): p. 1012-24.

62. Chen, H., et al., *Low serum levels of the innate immune component ficolin-3 is associated with insulin resistance and predicts the development of type 2 diabetes*. J Mol Cell Biol, 2012. 4(4): p. 256-7.

63. Zheng, B., et al., *Correlation between Ficolin-3 and Vascular Endothelial Growth Factor-to-Pigment Epithelium-Derived Factor Ratio in the Vitreous of Eyes with Proliferative Diabetic Retinopathy*. American Journal of Ophthalmology, 2011. 152(6): p. 1039-1043.

64. Szala, A., et al., *Ficolin-2 and ficolin-3 in women with malignant and benign ovarian tumours*. Cancer Immunology, Immunotherapy, 2013. 62(8): p. 1411-1419.
65. Shi, I., et al., *Aberrant signaling pathways in squamous cell lung carcinoma*. Cancer Inform, 2011. 10: p. 273-85.

66. Yao, M., et al., *The Clinical Significance of GP73 in Immunologically Mediated Chronic Liver Diseases: Experimental Data and Literature Review*. Clin Rev Allergy Immunol, 2018. 54(2): p. 282-294.

67. Xiong, J., et al., *Heat shock protein 70 downregulation inhibits proliferation, migration and tumorigenicity in hepatocellular carcinoma cells*. Oncol Lett, 2017. 14(3): p. 2703-2708.

68. Huang, J.T., et al., *Systematic Review and Meta-Analysis: Circulating miRNAs for Diagnosis of Hepatocellular Carcinoma*. J Cell Physiol, 2016. 231(2): p. 328-35.

69. Mou, T., et al., *Identification and interaction analysis of key genes and microRNAs in hepatocellular carcinoma by bioinformatics analysis*. World J Surg Oncol, 2017. 15(1): p. 63.