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Expression Profiles Analysis Identification and Interaction of Key Genes and Micrornas in Hepatocellular Carcinoma

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Research Article

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Abstract **Background:** Hepatocellular carcinoma is one of the most common malignant tumors, with rapid development and high malignancy. MicroRNAs have been reported to play important roles in hepatocellular carcinoma progression. **Aim:** To identify the key genes and miRNAs in HCC, and to explore their potential molecular mechanisms.
Methods: Gene expression profiles of GSE15471 (mRNA profile) and GSE57555 (miRNA profile) were downloaded from gene expression omnibus, which were analysed using R software and bioconductor packages. The gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichments of DEGs were performed using the DAVID database, and the protein–protein interaction networks of the DEGs were constructed from the STRING database. In addition, targets of differentially expressed miRNAs were predicted by the online resource miRDB. **Result:** In total, 191 differentially expressed genes were identified, including 142 upregulated and 49 downregulated genes. Functional analysis revealed that these DEGs were associates with wound healing, endodermal cell-cell adhesion, activation of MAPK activity and negative regulation of cell proliferation. In addition, we identified five DEMs, which were upregulated and downregulated. hsa-miR-122-5p may target the PDK4, and hsa-miR-21-5p probably targets SPOCK1 and PAIP2B. **Conclusions:** We applied integrated bioinformatics to identify key pathogenic genes involved in hepatocellular carcinoma and provide new clues for further studies of hepatocellular carcinoma.

Keywords: *Hepatocellular carcinoma, Gene expression omnibus, Gene ontology, Kyoto Encyclopedia of Genes and Genomes, Protein–protein interaction*

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide [1] with more than 0.55 million liver cancer cases annually [2,3]. The pathogenesis of HCC is a multistep process involving the multi-Step molecular pathogenesis [4] and cellular events of cancer metabolism and tumor microenvironment. [5,6]. Although the great advances of therapeutic approaches targeted at liver cancer, the recurrence and metastasis remain the major factors affecting the prognosis of patients with HCC [7]. It is urgent to investigate the molecular mechanisms involved in HCC initiation and progression to develop new treatment approaches. Therefore, bioinformatics analysis of microarray data has been widely used to developing biomarkers in malignancy such as HCC.

In recent years, a large number of studies have been conducted on differentially expressed genes (DEGs) in HCC, and their role in development and progression of HCC have been reported. [8,9]. And previous studies revealed that miRNAs

played a significant role in biological processes, molecular functions and different pathways of HCC [10,11], However, it remains unclear about the potential molecular pathway interactions between DEGs and miRNA in the pathogenesis of HCC. We analysed data generated by microarray technology to provides reliable molecular markers between DEGs and DEMs, as well as effective drug targets for treating HCC.

2. Materials and methods

2.1 Data source

The gene expression profiles between HCC and normal controls downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), GSE15471 and GSE57555. The GSE15471, which contains an mRNA expression profile of 39 HCC samples and 39 normal samples. The GSE57555 consist of an miRNA expression profile of 32 tumor samples and 32 normal samples.

2.2 Screening for DEGs and DEMs

R software was used to convert and discard the meaningless data. GEO2R was used to screen for miRNAs (differentially expressed miRNAs; DEMs) that were differentially expressed between tumor samples and normal samples. The adjusted $P < 0.05$ and $logFC \geq 2$ for DEGs and $P < 0.05$ and $logFC \geq 1$ for DEMs as a screening criteria. In this process, we needed the R package of limma in the Bioconductor package for identifying differentially expressed genes.

2.3 GO and KEGG analyses of DEGs

DAVID Database (https://david.ncifcrf.gov) is a bioinformatics database containing functional interpretation of lists of genes, where the analyses of GO and KEGG can be found. GO is a frequently-used bioinformatics tool because of providing comprehensive information about gene function of individual genomic products through ontology. Molecular functions (MF), biological processes (BP) and cellular components (CC) are three items of GO. KEGG is a set of high-throughput genes and protein pathways. The screening criteria of GO and KEGG analyses of DEGs identified in GSE15471 was $P < 0.05$, and the analyses were performed through DAVID online tools.

2.4 PPI network integration

The STRING database (http://string-db.org/) usually used to confirm the proteins and proteins interaction (PPI) of DEGs. Moreover, the Cytoscape software (http://www.cytoscape.org/) is a network for visualizing PPI network of common DEGs in HCC. Cytoscape Molecular Complex Detection (MCODE) plug-in was used to screen clustered sub-networks.

2.5 Prediction of microRNA targets

MiRDB (http://mirdb.org/miRDB/), a database of microRNA targets, was used to predicted the target genes for DEMs in GSE57555. The common genes were further screen by comparing the target genes with DEGs.

3. Results

3.1 Microarray data information and identification of DEGs in hepatocellular carcinoma

The results of the hepatocellular cancer expression microarray datasets GSE15471 was standardized as is shown in Figure 1A and Figure 1B. When the GSE15471 dataset was screened and obtained 191 DEGs by the limma package (corrected P-value<0.05, logFC >2), where 142 upregulated genes and 49 downregulated genes were identified (Table1). The heatmap of the top 100 DEGs are shown in Figure 2.

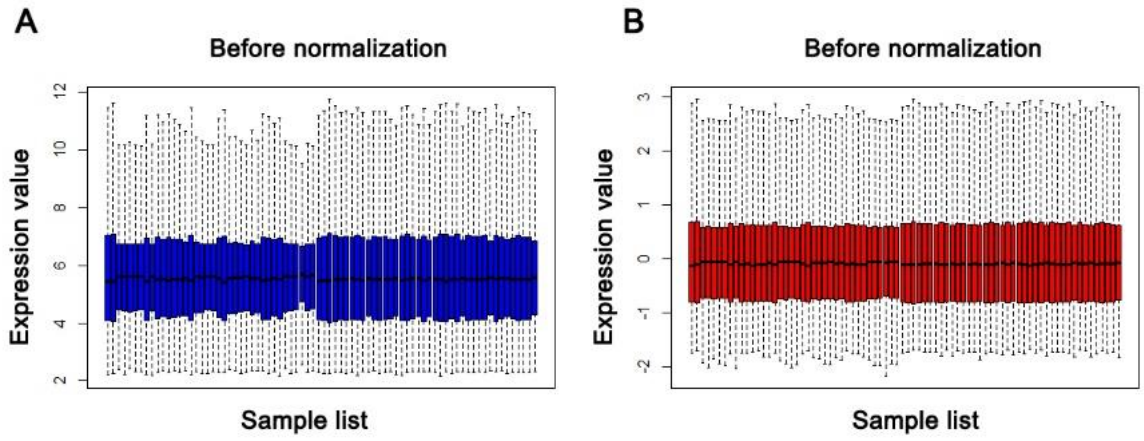

Figure 1. Standardization of gene expression. The standardization of GSE15471 data. A. The blue bar represents the data before normalization. B. The red bar represents the normalized data

Table 1. Screening DEGs in HCC by integrated microarray

Gene name (upregulated DEGs)
COL10A1 INHBA CTHRC1 SULF1 COL1A2 VCAN THBS2 COL11A1 GJB2 S100P COMP MMP1 FN1 COL5A2 CST1 FAP SFRP4 GREM1 CEACAM6 POSTN SLC6A14 MMP7 OLR1 GABRP RARRES1 COL3A1 OLFML2B COL5A1 ITGBL1 SFRP2 CXCL8 TCN1 TIMP1 GABBR1 /// UBD LOC102725271 /// NTM IGHG1 /// IGHG2 /// IGHM /// IGHV4-31 /// MIR8071-1 /// MIR8071-2 LCN2 GPRC5A FNDC1 MMP12 IGFBP3 RAB31 TMEM200A EDNRA CYP1B1 SHISA2 CTSE CXCL5 AEBP1 TNFAIP6 COL6A3 SLPI SFN CTSK DUOX2 GPNMB PLAT FCGR3A /// FCGR3B RGS1 OLFM4 DACT1 LY96 LOC101928916 /// NNMT MGC24103 ISLR SPARC SDR16C5 DKK1 MATN3 MFAP5 FAM19A5 CDH11 RP3-428L16.2 C15orf48 LOXL1 COL12A1 MOXD1 JUP /// KRT17 SULF2 PPAPDC1A TFF1 C19orf33 FBN1 KRT23 CCL20 CRISPLD1 MXRA5 BGN ALOX5AP SPOCK1 BCL2A1 TMC5 LAMP5 CXCR4 ZNF521 SEMA3C STEAP1 LOX COL8A1 CXCL6 ASPN LAMC2 CCL19 PLAU CLDN18 CAPG TMEM158 IFI27 COL4A1 MIR6787 /// SLC16A3 SPP1 CSTA MYOF PRRX1 NQO1 AK021804 LINC01094 PLXDC2 SLC16A4 LUM C3 NCF2 HTR2B CCDC109B NOX4 CFH /// CFHR1 HMCN1 C5AR1 FCGR1B LGALS1 CRP FCER1G CXCL3 ITGA2 PALLD ANO1 GLT8D2 ZFPM2 TMEM119 IFI44L CLEC5A HLA-DQA1 /// HLA-DQA2 /// LOC100509457

Gene name (downregulated DEGs)
ACTG1P4 /// AMY1A /// AMY1B /// AMY1C /// AMY2A /// AMY2B ALB AQP12A /// AQP12B AQP8 AZGP1 C5 CELA2A /// CELA2B CELA2B CELP CLPS CPA2 CTA-445C9.15 CTRC CTRL CUZD1 DPP10 EGF ERP27 FAM3B FGL1 GNMT GP2 GPHA2 KLK1 LGALS2 LOC101930067 PAIP2Bn PDK4 PLA2G1B PM20D1 PNLIPRP1 PNLIPRP2 PRSS3 PSAT1 RBPJL RNF186 RP11 320N7.2 SEL1L SERPINI2 SLC16A10 SLC43A1 SPX SYCN TEX11 TMED6 TMEM52 TPST2 TRHDE ZG16

3.2 Gene ontology and pathway enrichment analyses

GO and KEGG analyses to explore the function and pathways of the 191 identified DEGs were performed by using the DAVID. Figure 3A is the analysis of GO term enrichment, where we could see the biological processes category, the molecular function and the cell composition of upregulated genes and downregulated. As is shown in Figure 3B, in the biological process group, the upregulated genes were mainly enriched in extracellular matrix organization, cell adhesion, and proteolysis. The downregulated genes were mainly concentrated in heparan sulfate proteoglycan metabolic process. In the cell composition group, the upregulated genes were mainly enriched in extracellular space, extracellular exosomes, and extracellular matrix. The downregulated genes were mainly enriched in transport vesicle membrane, apical part of cell, and the collagen type V trimer. In the molecular function group, the upregulated genes were mainly enriched in calcium ion binding, serine-type endopeptidase activity, and extracellular matrix structural constituent. The downregulated genes were mainly enriched in extracellular matrix constituent conferring elasticity, oxidoreductase activity, acting on the CH-NH2 group of donors, oxygen as acceptor, N-acetylglucosamine-6-sulfatase activity, and cysteine-type endopeptidase inhibitor

activity. These results indicate that most DEGs are significantly enriched in extracellular substance, cell cycle regulation, and regulating endopeptidase activity.

The pathway analysis of KEGG showed that the pathway of extracellular matrix receptor interactions and focal adhesion had the most upregulated genes in seven pathways, meanwhile downregulated genes mainly took part in pancreatic secretion (Table 2).

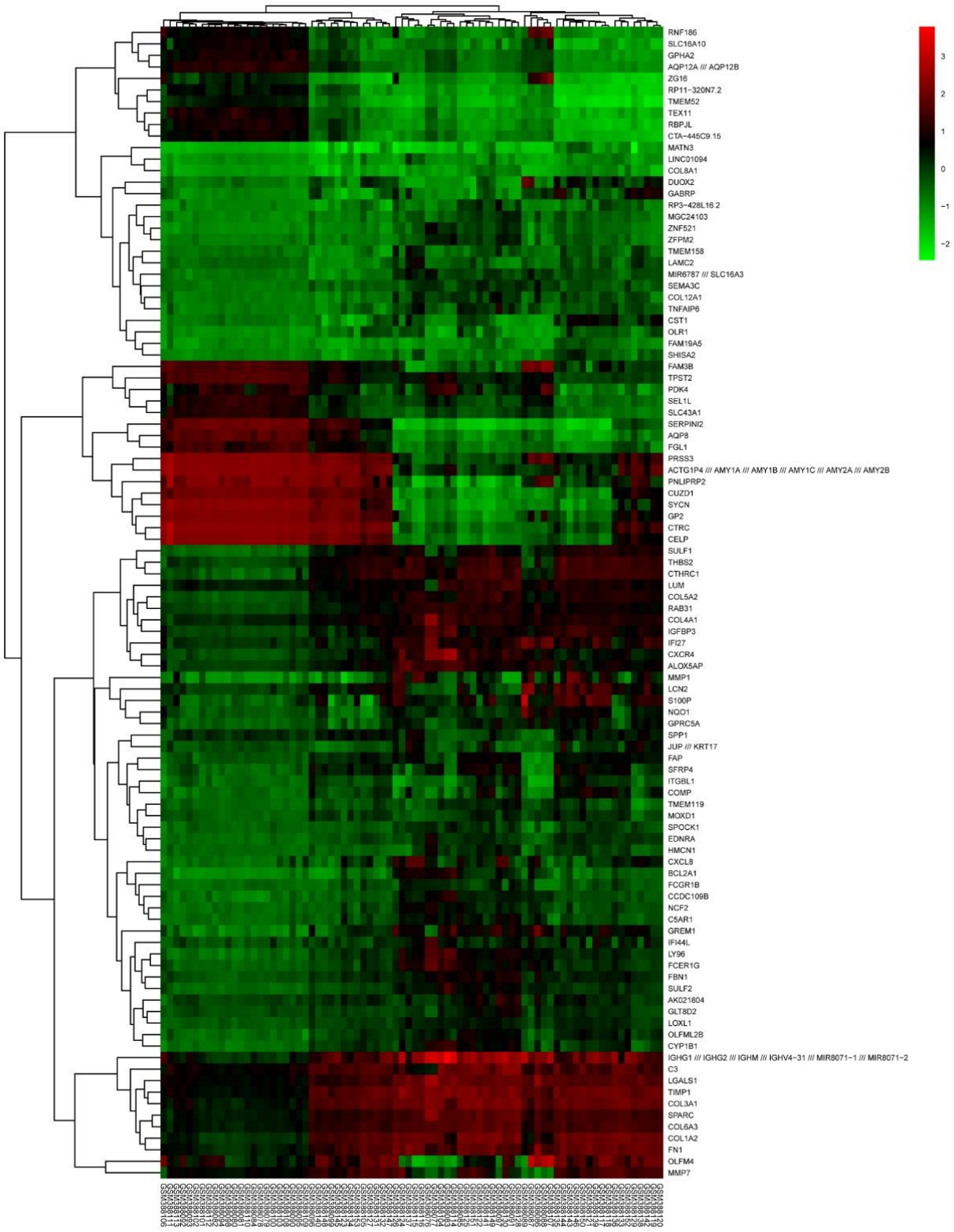

Figure 2. Heatmap of DEGs (top 100 genes) screened between HCC tissues and normal controls in GSE15471, $P < 0.05$ and log FC ≥ 2

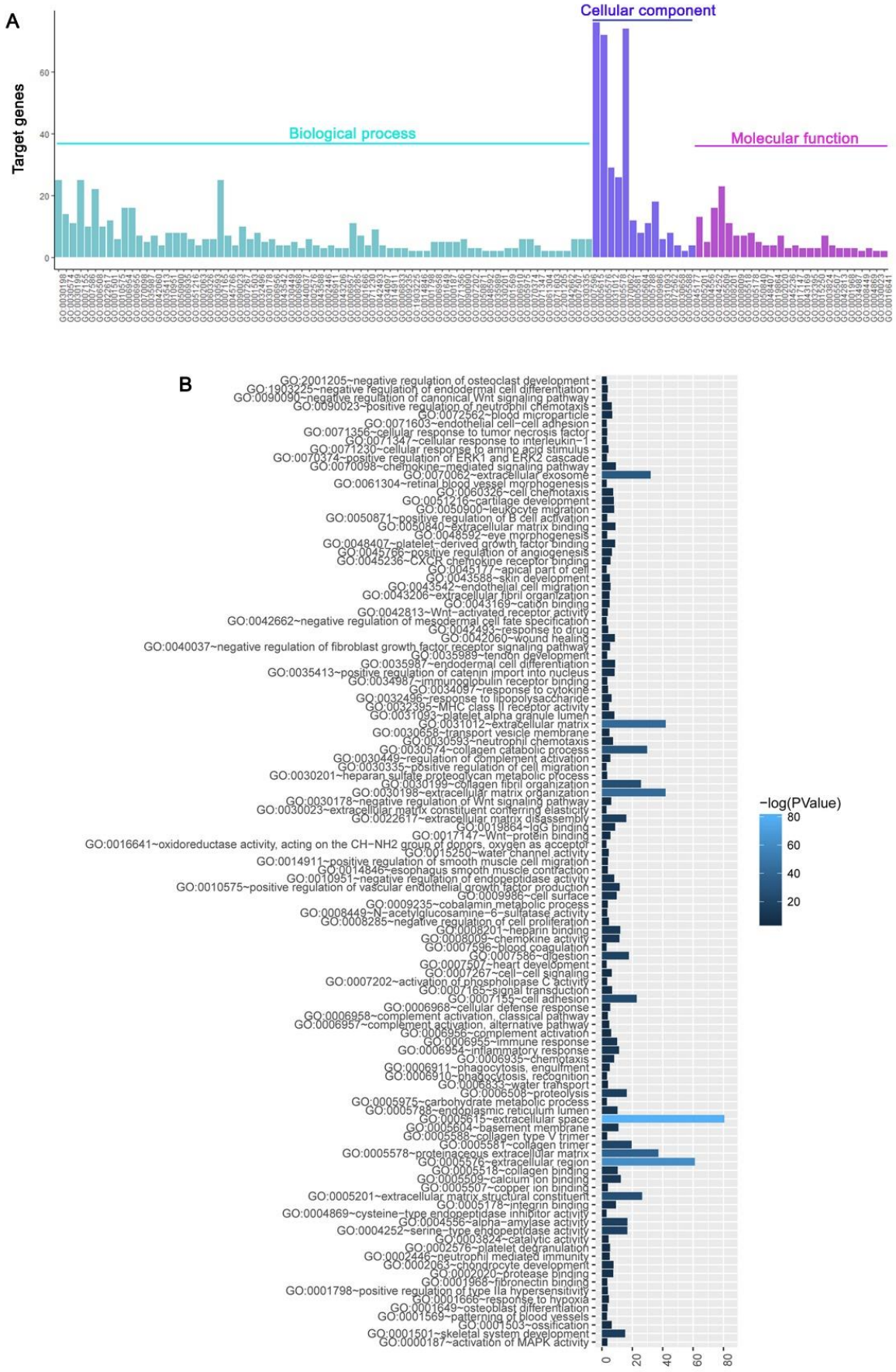

Figure 3. Functional enrichment analysis of DEGs in HCC. A. Go analyses divided DEGs according to their biological process, cellular component and molecular function B. GO enrichment significance items of DEGs in different functional groups

Table 2. KEGG pathway analysis of DEGs associated with ovarian cancer

Category		Term	Count*	*P*
Upregulated				
KEGG_PATHWAY	hsa04512	ECM-receptor interaction	13	< 0.01
KEGG_PATHWAY	hsa04510	Focal adhesion	13	< 0.01
KEGG_PATHWAY	hsa04974	Protein digestion and absorption	9	< 0.01
KEGG_PATHWAY	hsa05146	Amoebiasis	9	< 0.01
KEGG_PATHWAY	hsa05150	Staphylococcus aureus infection	7	< 0.01
KEGG_PATHWAY	hsa04145	Phagosome	10	< 0.01
KEGG_PATHWAY	hsa05323	Rheumatoid arthritis	8	< 0.01
Downregulated				
KEGG_PATHWAY	hsa04972	Pancreatic secretion	8	< 0.01
KEGG_PATHWAY	hsa00500	Starch and sucrose metabolism	5	< 0.01
KEGG_PATHWAY	hsa04974	Protein digestion and absorption	6	< 0.01
KEGG_PATHWAY	hsa04973	Carbohydrate digestion and absorption	5	< 0.01

3.3 Analyzing DEGs in hepatocellular cancer using a PPI network

In total, 191 nodes were mapped in the PPI network of identified DEGs (Fig. 4A). Which included 142 upregulated genes and 49 downregulated genes, and then 19 highest-scoring nodes were gained after screening, as shown in Figure 4B. The 19 significant genes showing significant interaction were LOX, MMP7, CTSK, ITGA2, TIMP1, COL3A1, SULF1, LUM, FNDC1, COL1A2, ISLR, COL6A3, CDH11, FAP, VCAN, SPARC, COMP, COL10A1 and COL8A1.

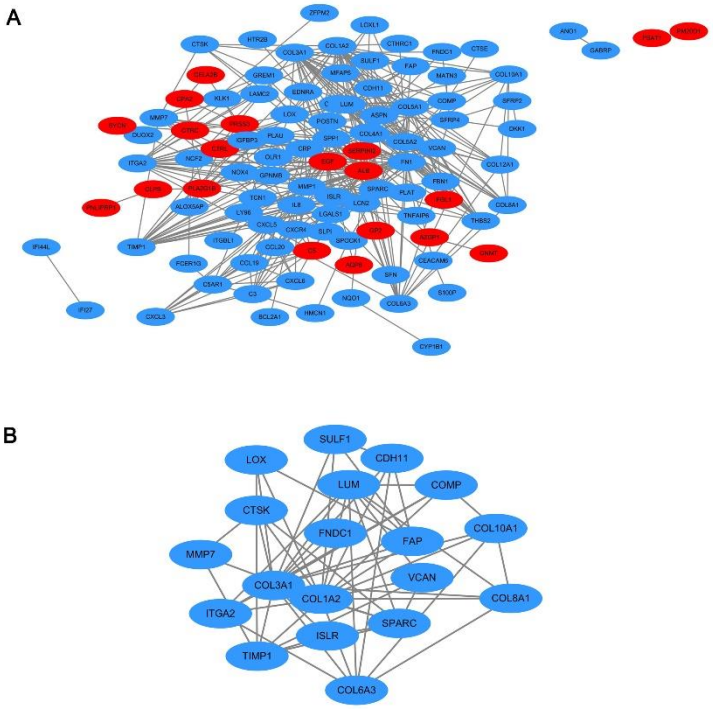

Figure 4. PPI network. A. PPI network of the common DEGs identified from GSE15471. B. One sub-networks was identified by Cytoscape MCODE plug-in.

3.4 MicroRNA–differentially expressed gene pairs

After analysing the GSE57555 dataset, Five DEMs that were differentially regulated between the HCC and the normal tissue samples were identified. Of these, three miRNAs were downregulated and two miRNAs were upregulated. The

miRDB database was used to predict target genes of the identified DEMs (Table 3). We screened genes with an opposing expression trend depend on the target genes with DEGs from GSE15471, this was all for further analysis. In the downregulated genes, hsa-miR-23a-3p probably targets VCAN, TNFAIP6, SPOCK1, SLC6A14, PLAU, MFAP5, FBN1, CRISPLD1 and COLHA1, while hsa-miR-200b-3p potentially targets ZFPM2, SULF1, GREM1, FN1 and CDH11, and hsa-miR-21-5p may target SPOCK1, CCL20, ASPN. At the same time, in the upregulated genes, hsa-miR-122-5p may target PDK4, but meanwhile has-miR-4286 may target ITGBL1, CYP1B1 and Col5A2.

Table 3. Differentially expressed microRNAs in HCC and their potential target genes.

			Target Genes	
miRNA	*P*	Log FC	Upregulated	Downregulated
hsa-miR-122-5p	0.001	8.27		PDK4
hsa-miR-4286	0.003	1.63	ITGBL1, CYP1B1, Col5A2	
hsa-miR-23a-3p	0.02	-1.09	VCAN, TNFAIP6, SPOCK1, SLC6A14, PLAU, MFAP5, FBN1, CRISPLD1, COLHA1	PDK4
hsa-miR-200b-3p	0.01	-1.12	ZFPM2, SULF1, GREM1, FN1, CDH11	TRHDE, PSAT1
hsa-miR-21-5p	0.0002	-4.32	SPOCK1, CCL20, ASPN	PAIP2B

Discussion

The occurrence and development of HCC is a complex and multistep process that relates to a variety of cumulative genetic and epigenetic changes (12, 13). In this work, 142 up-regulated genes and 49 down-regulated genes were screened out. The top 10 most significantly upregulated genes were COL10A1 INHBA CTHRC1 SULF1 COL1A2 VCAN THBS2 COL11A1 GJB2 S100P. The top 10 most significantly downregulated genes were CTRL SERPINI2 AQP8 ALB SYCN TMED6 GNMT CELA2B PNLIPRP2 ERP27. In addition, the upregulated genes were mainly involved in the cell adhesion, inflammatory response, extracellular exosome, heparin binding, wound healing and apoptotic process that the downregulated genes were mainly involved in the activation of MAPK activity. MAPK, a family of serine/threonine kinases, play a significant role in the tumor proliferation, differentiation, migration, apoptosis and inflammation (14, 15).

There is now well-established in the literature that miRNAs play a vital role in regulating the translation and degradation of mRNAs (16, 17). More and more research showed that miRNAs played an important role in cell death, cell proliferation, stress and resistance. In the present work, we identified 3 upregulated DEM and 2 downregulated DEM. We identified potential targets of these DEMs by comparing target genes with DEGs.

The upregulated DEM, hsa-miR-122-5p, potentially targets PDK4. PDK4 is a key mitochondrial enzyme of PDK family that is involved in regulating the switch to aerobic glycolysis by shunting pyruvate metabolism from the mitochondria to the cytoplasm for glycolysis (18, 19), and it may play an important role between cellular respiration and cell cycle progression as a prime molecular suspect in crosstalk (20). It was reported that PDK4 in hepatocellular carcinoma cells could be suppressed by the miR-129-5p (21), but the role of hsa-miR-122-5p in the regulation of CCR7 in hepatocellular carcinoma pathogenesis still needs further study.

The downregulated DEM, hsa-miR-21-5p, potentially targets the SPOCK1 and SPAI2B. SPOCK1-mediated cell proliferation and migration influence the survival of several tumors, such as non-small cell lung cancer, gastric cancer and pancreatic cancer (22-24) PAIP2 is a translational repressor capable of inhibiting cap-dependent translation that has been implicated in multiple biological processes, including cell proliferation and differentiation (25, 26). However, whether SPOCK1 and PAIP2 are involved in the HCC proliferation and migration is unclear.

In conclusion, our study has profiled DEGs using bioinformatics analyses and to find potential miRNAs to improve our understanding of the pathogenesis of HCC. In this work, 191 DEGs and 5 DEMs were screened. Given their interactions

with PDK4 SPOCK1 and PAIP2, respectively, the role of hsa-miR-122-5p and hsa-miR-21-5p in HCC may provide effective targets for the treatment of HCC. However, further molecular biological experiments studies are required to confirm the function of the identified genes obtained from bioinformatics analysis.

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Conflict of interest

Authors have no conflict of interest

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