Identification of Hepatocellular Carcinoma-Related Potential Genes and Pathways Through Bioinformatic-Based Analyses

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Background: Hepatocellular carcinoma (HCC) is one of the most prevalent tumors worldwide. However, the key roles of most molecules associated with tumor proliferation, invasion, and metastasis in HCC remain unclear. It is therefore important to explore potential mechanisms underlying tumorigenesis and to screen genes and pathways identified from such research for their role in pathogenesis.

Materials and Methods: We selected microarray data GSE62043 consisting of paired tissue samples from 100 HCC patients, then these data were analyzed to identify differentially expressed genes (DEGs). Next, gene ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were carried out to elucidate the biological processes, molecular function, cellular component (CC), and KEGG signaling pathways for the DEGs. We then constructed protein–protein interaction (PPI) networks, followed by a functional enrichment analysis, from which we obtained two significant gene modules. Finally, the gene expression data obtained from this test set were subjected to validation studies using an independent set of hepatocellular patient data archived in The Cancer Genome Atlas and Genotype-Tissue Expression (TCGA/GTEX) database.

Results: A total of 425 DEGs were identified that met both of our criteria for significance: (1) a $|\log_2\text{-fold change (FC)}| \geq 1.2$ and (2) an adjusted p value < 0.01 . From these data, two significant gene modules, containing 28 pathway-related hub genes, were identified.

Conclusion: Through application of a test/validation algorithm using HCC datasets from two independent databases, we identified a number of genes that could serve as potential biomarkers for the molecular diagnosis and therapeutic intervention of HCC, including the known genes, *IGF1*, *IGF2*, *NDC80*, *CDK1*, *CENPF*, *CDCA8*, *CCNB1*, *BIRC5*, *NCAPG*, and *CDCA5*, and the novel genes, *CENPU* and *SPC25*, which are associated with cell cycle, mitotic cell cycle, and organelle organization.

Keywords: differentially expressed genes, bioinformatics, hepatocellular carcinoma

Introduction

HEPATOCELLULAR CARCINOMA (HCC) is the most common form of primary liver cancer and is a leading cause of cancer-related deaths worldwide (Balogh *et al.*, 2016). HCC occurs frequently in persons with chronic liver diseases such as cirrhosis caused by independent risk factors, including chronic hepatitis B virus or hepatitis C virus infection (Balogh *et al.*, 2016).

An early diagnosis is essential for obtaining the best treatment results for HCC patients.

Patients suffering from late-stage HCC have a poor prognosis with limited therapeutic options. α -Fetoprotein (*AFP*),

which is often expressed at high levels in HCC patients, has historically been used as a clinical biomarker (Chen *et al.*, 1984). Currently, there is a substantial body of research concerning HCC-specific targeted therapies. These studies have mainly focused on the serine–threonine kinases, *Raf-1* and B-Raf, and the receptor tyrosine kinase activity of vascular endothelial growth factor receptors (*VEGFR-1*, *VEGFR-2*, and *VEGFR-3*) and platelet-derived growth factor receptor β (*PDGFR- β*) (Wilhelm *et al.*, 2004; Chang *et al.*, 2007). Although the prognosis for metastatic or unresectable HCC has improved in recent years due to the approval of the target drug, Sorafenib (Llovet *et al.*, 2008), the overall survival rate is still poor. This suggests that the mechanisms underlying HCC

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progression require further exploration, which would hopefully lead to the discovery of novel therapeutic targets.

Gene chip microarrays can provide genome-wide expression profiles (Lee *et al.*, 2013) that can be analyzed using unbiased bioinformatic tools to discover new therapeutic targets. Exporting data from these analyses into modern pathway profiling software could uncover meaningful clues toward novel understanding (Guo *et al.*, 2017). Vast datasets have been developed from HCC patient specimens in recent years (Wang *et al.*, 2015; Mou *et al.*, 2017), and a lot of differentially expressed genes (DEGs) have been identified and characterized using gene ontology (GO) and signaling pathway analyses (Zhao *et al.*, 2019).

In this study, we downloaded the GSE62043 mRNA expression profiles from the website of Gene Expression Omnibus (GEO). GEO is a public online database for uploading, archiving, retrieving, and querying microarray

datasets. DEG analyses were carried out between primary tumor tissues (marked tumor) of HCC patients and paired, adjacent nonmalignant tissues (marked control) with R software (Dessau and Phipper, 2008) using the built-in *limma et al.* packages (Smyth, 2005). Then, functional and signaling pathway enrichment analyses were performed with the online bioinformatics resource DAVID. We also constructed protein–protein interaction (PPI) networks of the DEGs and identified those genes that appeared in two or more analyses (module genes). Finally, we validated these candidate module genes using separate datasets available through The Cancer Genome Atlas and Genotype-Tissue Expression (TCGA/GTEx) databases. Using this approach, we identified several potentially important HCC-related genes and pathways for further study to help define their roles in diagnosis, prognosis, and therapeutic treatment.

TABLE 1. FOUR HUNDRED TWENTY-FIVE DIFFERENTIALLY EXPRESSED GENES WERE IDENTIFIED IN GSE62043, INCLUDING 48 UPREGULATED GENES AND 377 DOWNREGULATED GENES IN 100 PAIRED SAMPLES OF HCC PATIENTS

DEGs	Gene name
Upregulated genes	<i>SPINK1, CENPF, BIRC5, NCAPG, MDK, CCNB1, CDKN3, SQLE, TK1, OIP5, UBE2T, NDC80, CDCA5, CDCA8, FAM83D, KIAA0101, STMN1, SPC25, COX7B2, TOP2A, CDKN2A, CDT1, CDK1, GNAZ, MAGEA2B, ESM1, IGF2BP3, EZH2, HOXA13, CAP2, AKR1B1, PRC1, ENAH, TYMS, CENPU, MND1, CDCA2, LCN2, SPON2, CGREF1, APLN, SULT1C2, MAGEA12, SMPX, CDC6, CEP55, CLGN, AKR1C3</i>
Downregulated genes	<i>GCKR, SAMSNI, FCN1, SYNE1, METRNL, NDRG2, VCAM1, MTHFD1, NGFR, GPD1, GLUD2, RIDA, KDM8, IDNK, COL3A1, NR4A2, SLC1A2, FAM102A, TKFC, FGL2, EPB41L4B, CLIC6, CDKN1C, DHODH, MIR22HG, GMFG, ADAMTSL3, ACP5, PTGIR, SGK1, UGP2, IL18RAP, SVEP1, STAB1, KLC4, ALDH1B1, GLRA3, OTC, IER2, EFEMP1, MTP, PON3, GNE, CD81, CPB2, SLC05A1, MMP19, GZMK, ACADSB, SERTAD1, SLC17A3, CAT, ANG, APOC4, CMBL, FAM65C, C1RL, EMILIN1, PRKCB, DLC1, SLC38A2, AREG, DUSP5, UGT2B4, CYP2D6, NTN4, SPINT2, IGKC, C1QB, SLC38A4, CSF1R, CDO1, RGN, FGA, FGG, ACAT1, AEBP1, TPPP2, RNF125, EDNRB, GSTP1, DAO, COLEC10, AKAP2, GSTA2, ATOH8, TRPM8, TRIB1, ATF5, Hsp40, OBSL1, AQP9, SERPINA3, GSTA1, SLC1A1, UGT2B11, TAT, FHL2, TSLP, KCNJ16, CCL3, CPXM2, GLDC, CDH1, SIK1, FMO3, GSTZ1, ADAMTSL2, SULT1A1, APOA1, A1BG, SMPD3, PLPP3, GPT2, ANGPTL4, DACT3, ACOT12, IL10RA, AQP3, PLK3, ADAMTS1, HHIP, HPGD, RBP1, ITGAD, HAO1, ID2, NAMPT, PLIN2, CILP, CP, ABAT, PPARGC1A, DUSP6, GSTA3, NPC1L1, HBA1, FPR1, AXL, ACSM3, SIGLEC11, MYC, UGT2B17, HAND2-AS1, ALDH2, AOX1, SLC27A5, PROZ, AGTR1, FETUB, FCGR3A, NR0B2, MFSD2A, GABARAPL1, FXYD1, B3GAT1, F11, RGS2, SERPINA7, ANGPTL3, POU2AF1, TMSB4Y, AR, BEX1, FMOD, TEK, QSOX1, ETNPPL, IGF1, ERFF1, ASPA, HSD17B6, SLC22A10, PBLD, SULF2, MS4A7, HMOX1, BASP1, FBLN5, FEZ1, DUSP1, CLDN10, IL7R, A2M, KLF2, ACSL1, LCAT, ETS2, ZGPAT, ADH6, PON1, ARG1, GRASP, SLC27A2, NTF3, HPGDS, ALDH6A1, OGDHL, ATF3, FGB, CCL4, COTL1, DMGDH, SLC18A1, SERPINA4, AGXT2, ANGPTL1, MASP1, JUNB, UROC1, MS4A6A, IL33, KMO, MAT1A, PAMR1, CIR, PCK1, HP, GEM, FAM13A, SLC10A1, GCGR, GRAMD1C, TTR, AADAT, TMEM45A, ESR1, GADD45B, PZP, DEPDC7, LHX2, GDF2, FGFR2, PTN, CSRNPI, FAM151A, SKAP1, LILRB5, CTH, ADRA1B, SHBG, ADGRG7, HRG, CFD, ZFP36, RSP03, SLC01B1, PTH1R, UGT2B7, MYOM2, ENO3, HPD, C8B, SOCS3, VNN1, G6PC, CDHR2, GHR, ALDOB, ALB, NR4A1, HPX, MT1H, CCL14, RCAN1, ASS1, IGFBP1, NNMT, MAN1C1, VSIG4, IGHAI, EGR2, HSD17B2, TACSTD2, ALDH8A1, KCNN2, FBP1, PDE2A, RDH16, IGHV4-31, MT2A, LECT2, PRG4, FOLH1, ANGPTL6, KLKB1, ASPN, SIPA1, SFRP1, MPPED1, MTIX, SERPINA11, TNXA, MT1P3, FCGR2B, BCHE, GPM6A, CFTR, SOCS2, CYR61, CD163, CDH19, TFPI2, GLYATL1, CCL19, C8A, BBOX1, ANK3, CETP, S100A8, MBL2, MT1HL1, FOSB, ZG16, STAB2, BCO2, ECM1, CYP2C9, IGFBP3, NR4A3, DBH, CYP39A1, DEFA3, SLC25A47, MT1G, MT1B, CYP2C19, CXCL2, DPT, IL1RAP, IGF2, PDGFRA, PLGLB2, SDS, NAT2, RND3, AKR1D1, LYVE1, HBB, FAM134B, C7, CYP2A6, CLRN3, JCHAIN, ID1, SLC22A1, CFP, ADAMTS13, HAO2, MT1F, SPP2, COLEC11, EGR1, TMEM27, CYP26A1, GBA3, CYP1A2, CYP2A7, FOS, CD5L, CYP2C8, THRSP, SRD5A2, MFAP4, SLC01B3, HAMP, DNASE1L3, MARCO, CXCL12, ADH4, IGFALS, CLEC1B, FCN3, CLEC4G, CRHBP, FCN2, CXCL14</i>

The differentially expressed genes are listed from the smallest to the largest adjusted *p* value.
DEG, differentially expressed gene; HCC, hepatocellular carcinoma.

FIG. 1. GO terms and KEGG pathways of DEGs significantly enriched in HCC. DEG, differentially expressed gene; GO, gene ontology; HCC, hepatocellular carcinoma; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Materials and Methods

Microarray data and data preprocessing

Data from the Agilent microarray file GSE62043 (Thurnherr *et al.*, 2016) were downloaded from the public NCBI-GEO database (Edgar *et al.*, 2008; Barrett *et al.*, 2009) and executed on the GPL6480 platform. GSE62043 contains 100 paired primary and adjacent nonmalignant tissue samples from HCC patients. After the normalized log₂ ratio (Cy5/Cy3) representing tumor/normal of the GSE62043 dataset, normalized by the loess and quantile method (Smyth and Speed, 2003), was downloaded, probe identification numbers were converted to gene symbols using as a reference the Whole Human Genome Microarray 4×44K G4112F (Probe Name Version). When multiple probes corresponded to the same gene, the probe with the greatest *p* value from the downstream differential analysis was chosen to determine the differential gene expression value.

Identification of DEGs

DEG analysis refers to identification of genes that are identified as being expressed at significantly different levels by multiple modes of analysis (Korpelainen and Tuimala, 2014). We used *limma* (Smyth, 2005) and R (Dessau and Pipper, 2008) packages to identify the DEGs in primary tumor tissues (marked tumor) of HCC patients and compared them with adjacent nonmalignant tissues (marked normal) of HCC patients. Genes with |log₂-fold change (FC)| ≥ 1.2 and adjusted *p* values < 0.01 (moderated *t*-statistics, corrected by Benjamini and Hochberg's method)

were used in the next analysis stage (Benjamini and Hochberg, 1995).

GO enrichment and pathway analysis

The GO (Ashburner *et al.*, 2000) is a structured vocabulary of terms describing gene products according to the biological process (BP), molecular function (MF), and cellular component (CC). The Kyoto Encyclopedia of Genes and Genomes (KEGG) (Ogata *et al.*, 1999) provides data resources of known biological metabolic pathways. We used DAVID (Dennis *et al.*, 2003), a web-accessible program that integrates functional genomic annotations with intuitive graphical summaries, to view the GO and KEGG enrichment of DEGs; a *p* value < 0.05 was considered statistically significant.

PPI networks and module analysis

The Search Tool for the Retrieval of Interacting Genes (STRING, Version 11.0) (Franceschin *et al.*, 2013) database was used to retrieve DEGs' encoded protein and PPI network information. This database contains >24.6 million proteins and 2 billion interactions involved in 5,090 organisms. We uploaded the DEGs into the STRING database and set the interaction score ≥ 0.900 (highest confidence) as the significant threshold. Then, the PPI networks were constructed using Cytoscape (Kohl *et al.*, 2011; Smoot *et al.*, 2011) software. The Molecular Complex Detection (MCODE) built in Cytoscape is an automated method that was used to analyze highly interconnected modules as molecular complexes or clusters. The analysis parameters were set by default. The functional enrichment analysis was executed for DEGs, from

TABLE 2. THE TOP FIVE PATHWAYS IN GO AND KEGG ENRICHMENT ANALYSIS OF DEGs

Category	Term	Count	p Value
Upregulated DEGs			
GOTERM_BP_FAT	GO:1903047 ~ mitotic cell cycle process	22	3.03E-15
GOTERM_BP_FAT	GO:0051301 ~ cell division	19	4.31E-15
GOTERM_BP_FAT	GO:0007059 ~ chromosome segregation	16	7.26E-15
GOTERM_BP_FAT	GO:0022402 ~ cell cycle process	25	8.33E-15
GOTERM_BP_FAT	GO:0000278 ~ mitotic cell cycle	22	1.59E-14
GOTERM_CC_FAT	GO:0000775 ~ chromosome, centromeric region	9	1.01E-07
GOTERM_CC_FAT	GO:0000793 ~ condensed chromosome	9	2.27E-07
GOTERM_CC_FAT	GO:0005694 ~ chromosome	15	4.96E-07
GOTERM_CC_FAT	GO:0098687 ~ chromosomal region	10	9.25E-07
GOTERM_CC_FAT	GO:0044427 ~ chromosomal part	13	5.38E-06
GOTERM_MF_FAT	GO:0003682 ~ chromatin binding	7	0.001513775
GOTERM_MF_FAT	GO:0019899 ~ enzyme binding	11	0.01131879
GOTERM_MF_FAT	GO:0044877 ~ macromolecular complex binding	9	0.014605682
GOTERM_MF_FAT	GO:0019900 ~ kinase binding	6	0.016144185
GOTERM_MF_FAT	GO:0042802 ~ identical protein binding	9	0.018143497
KEGG_PATHWAY	hsa04110: Cell cycle	4	0.002696712
KEGG_PATHWAY	hsa04115: p53 signaling pathway	3	0.010269564
KEGG_PATHWAY	hsa05206: MicroRNAs in cancer	4	0.026640176
Downregulated DEGs			
GOTERM_BP_FAT	GO:0019752 ~ carboxylic acid metabolic process	76	1.24E-27
GOTERM_BP_FAT	GO:0043436 ~ oxoacid metabolic process	76	1.82E-27
GOTERM_BP_FAT	GO:0006082 ~ organic acid metabolic process	79	3.25E-27
GOTERM_BP_FAT	GO:0032787 ~ monocarboxylic acid metabolic process	56	6.95E-22
GOTERM_BP_FAT	GO:0009605 ~ response to external stimulus	105	4.94E-18
GOTERM_CC_FAT	GO:0005615 ~ extracellular space	97	8.91E-23
GOTERM_CC_FAT	GO:0005576 ~ extracellular region	191	7.67E-22
GOTERM_CC_FAT	GO:0044421 ~ extracellular region part	169	8.99E-21
GOTERM_CC_FAT	GO:0070062 ~ extracellular exosome	128	8.30E-16
GOTERM_CC_FAT	GO:1903561 ~ extracellular vesicle	128	1.18E-15
GOTERM_MF_FAT	GO:0048037 ~ cofactor binding	26	2.09E-09
GOTERM_MF_FAT	GO:0005102 ~ receptor binding	68	6.11E-09
GOTERM_MF_FAT	GO:0043168 ~ anion binding	25	1.98E-08
GOTERM_MF_FAT	GO:0042802 ~ identical protein binding	60	3.67E-07
GOTERM_MF_FAT	GO:0031406 ~ carboxylic acid binding	17	7.52E-07
KEGG_PATHWAY	hsa00982: Drug metabolism—cytochrome P450	18	2.36E-11
KEGG_PATHWAY	hsa05204: Chemical carcinogenesis	17	3.26E-09
KEGG_PATHWAY	hsa04610: Complement and coagulation cascades	15	2.67E-08
KEGG_PATHWAY	hsa01100: Metabolic pathways	73	4.06E-08
KEGG_PATHWAY	hsa00830: Retinol metabolism	14	8.41E-08

GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

which two significant modules of genes were identified with $p < 0.05$ set as the threshold.

Validation of gene expression and overall survival of module genes

Gene Expression Profiling Interactive Analysis (Tang *et al.*, 2017) is a web sever, which contains the TCGA and GTEx datasets. First, we uploaded the genes identified by the PPI networks to validate the consistency of gene expression between the GSE62043 and TCGA/GTEx liver hepatocellular carcinoma (LIHC) datasets; we set the parameters, $|\log_2FC|$ cutoff: 1.2 and p value cutoff: 0.01, as the threshold criteria. Next, we performed the overall survival analysis. The patients in the TCGA/GTEx dataset were divided into high and low expression groups using the median transcripts per kilobase million (TPM) as a breakpoint, and significance was determined using a log-rank test with $p < 0.05$. The functional enrichment

analysis was executed to annotate the final potential key genes with the filter parameter, p value $< 1 \times 10^{-10}$, as the threshold in the GenCLiP 2.0 website (Wang *et al.*, 2014).

Results

Identification of DEGs

In this study, we identified DEGs from 100 paired primary tumor tissues (tumor) using limma packages, compared with adjacent nonmalignant tissues (control). Using both $|\log_2\text{-fold change (FC)}| \geq 1.2$ and adjusted p value < 0.01 criteria, a total of 425 genes were identified, including 48 upregulated genes and 377 downregulated genes (Table 1).

GO enrichment and pathway analysis

To further explore potential targets of these DEGs in HCC, we performed GO and pathway analyses on HCC with the

FIG. 2. PPI networks of DEGs. **(A)** Based on the STRING online database, 211 genes/nodes were filtered into the DEG PPI network. The two *highlighted circle areas* are the most significant modules. **(B)** The most significant module 1 from the PPI network (all genes are downregulated genes and labeled *light gray*). **(C)** The second significant module 2 from the PPI network (all genes are upregulated genes and labeled *dark gray*). PPI, protein–protein interaction.

B

FIG. 2. (Continued).

C

TABLE 3. FUNCTIONAL AND PATHWAY ENRICHMENT OF MODULE 1 GENES

Category	Term	Count	p Value	Genes
GOTERM_BP_FAT	GO:0002576 ~ platelet degranulation	13	4.67E-24	A2M, CFD, QSOX1, FGB, IGF1, FGG, IGF2, APOA1, ALB, HRG, FGA, A1BG, SPP2
GOTERM_BP_FAT	GO:0045055 ~ regulated exocytosis	13	9.72E-19	A2M, CFD, QSOX1, FGB, IGF1, FGG, IGF2, APOA1, ALB, HRG, FGA, A1BG, SPP2
GOTERM_BP_FAT	GO:0006887 ~ exocytosis	13	5.87E-17	A2M, CFD, QSOX1, FGB, IGF1, FGG, IGF2, APOA1, ALB, HRG, FGA, A1BG, SPP2
GOTERM_CC_FAT	GO:0034774 ~ secretory granule lumen	13	1.49E-24	A2M, CFD, QSOX1, FGB, IGF1, FGG, IGF2, APOA1, ALB, HRG, FGA, A1BG, SPP2
GOTERM_CC_FAT	GO:0060205 ~ cytoplasmic membrane-bounded vesicle lumen	13	1.89E-23	A2M, CFD, QSOX1, FGB, IGF1, FGG, IGF2, APOA1, ALB, HRG, FGA, A1BG, SPP2
GOTERM_CC_FAT	GO:0031983 ~ vesicle lumen	13	2.13E-23	A2M, CFD, QSOX1, FGB, IGF1, FGG, IGF2, APOA1, ALB, HRG, FGA, A1BG, SPP2
GOTERM_MF_FAT	GO:0005102 ~ receptor binding	10	1.73E-06	A2M, FGB, IGF1, FGG, IGF2, APOA1, HRG, FGA, IGFBP1, CYR61
GOTERM_MF_FAT	GO:0019838 ~ growth factor binding	4	2.34E-04	A2M, IGFBP3, IGFBP1, CYR61
GOTERM_MF_FAT	GO:0005520 ~ insulin-like growth factor binding	3	3.27E-04	IGFBP3, IGFBP1, CYR61
KEGG_PATHWAY	hsa04610: Complement and coagulation cascades	5	1.12E-06	A2M, CFD, FGB, FGG, FGA
KEGG_PATHWAY	hsa04611: Platelet activation	3	0.011696553	FGB, FGG, FGA
KEGG_PATHWAY	hsa05150: <i>Staphylococcus aureus</i> infection	2	0.068510167	CFD, FGG

criterion of p value <0.05 (Fig. 1). As shown in Figure 1, it shows the top six significant terms for each of the following: the BP, CC, MF, and KEGG pathways of DEGs, respectively.

We also show annotation of the upregulated genes as well as downregulated genes. As shown in Table 2, in the BP group, the upregulated DEGs were mainly enriched for genes in-

involved in the mitotic/cell cycle processes, cell division, and chromosome segregation. The downregulated DEGs were enriched genes in the carboxylic acid metabolic processes, oxoacid metabolic process, and other organic acid metabolic processes. In the CC group, the upregulated DEGs were mainly enriched for genes associated with the chromosome,

TABLE 4. FUNCTIONAL AND PATHWAY ENRICHMENT OF MODULE 2 GENES

Category	Term	Count	p Value	Genes
GOTERM_BP_FAT	GO:0000819 ~ sister chromatid segregation	10	1.21E-16	CDCA5, CEP55, BIRC5, CCNB1, NCAPG, CENPU, CDCA8, CENPF, NDC80, SPC25
GOTERM_BP_FAT	GO:0098813 ~ nuclear chromosome segregation	10	1.17E-15	CDCA5, CEP55, BIRC5, CCNB1, NCAPG, CENPU, CDCA8, CENPF, NDC80, SPC25
GOTERM_BP_FAT	GO:0007059 ~ chromosome segregation	10	4.64E-15	CDCA5, CEP55, BIRC5, CCNB1, NCAPG, CENPU, CDCA8, CENPF, NDC80, SPC25
GOTERM_CC_FAT	GO:0098687 ~ chromosomal region	9	4.13E-12	CDCA5, BIRC5, CCNB1, CDK1, CENPU, CDCA8, CENPF, NDC80, SPC25
GOTERM_CC_FAT	GO:0000775 ~ chromosome, centromeric region	8	6.56E-12	CDCA5, BIRC5, CCNB1, CENPU, CDCA8, CENPF, NDC80, SPC25
GOTERM_CC_FAT	GO:0000793 ~ condensed chromosome	8	1.38E-11	CDCA5, BIRC5, CCNB1, NCAPG, CENPU, CENPF, NDC80, SPC25
GOTERM_MF_FAT	GO:0035173 ~ histone kinase activity	2	0.006123483	CCNB1, CDK1
GOTERM_MF_FAT	GO:0003682 ~ chromatin binding	3	0.009912687	CDCA5, CDK1, CENPF
GOTERM_MF_FAT	GO:0004693 ~ cyclin-dependent protein serine/threonine kinase activity	2	0.010936591	CCNB1, CDK1
KEGG_PATHWAY	hsa04115: p53 signaling pathway	2	0.028939863	CCNB1, CDK1
KEGG_PATHWAY	hsa04914: Progesterone-mediated oocyte maturation	2	0.037469108	CCNB1, CDK1
KEGG_PATHWAY	hsa04114: Oocyte meiosis	2	0.047638096	CCNB1, CDK1

TABLE 5. VALIDATION OF THE GENE EXPRESSION CHANGES OF TWO MODULES BETWEEN GSE62043 AND TCGA/GTEX

Gene	HCC patients (GSE62043)	LIHC patients (TCGA/GTEX)
Module 1		
<i>IGFBP3</i>	Down	Down
<i>IGFBP1</i>	Down	—
<i>A2M</i>	Down	—
<i>IGF2</i>	Down	Down
<i>IGF1</i>	Down	Down
<i>QSOX1</i>	Down	—
<i>ALB</i>	Down	—
<i>FGA</i>	Down	—
<i>FGG</i>	Down	—
<i>CYR61</i>	Down	Down
<i>SPP2</i>	Down	—
<i>CP</i>	Down	—
<i>APOA1</i>	Down	—
<i>HRG</i>	Down	—
<i>A1BG</i>	Down	—
<i>FGB</i>	Down	—
<i>CFD</i>	Down	—
Module 2		
<i>NDC80</i>	Up	Up
<i>CDK1</i>	Up	Up
<i>CENPF</i>	Up	Up
<i>CDCA8</i>	Up	Up
<i>CCNB1</i>	Up	Up
<i>BIRC5</i>	Up	Up
<i>CEP55</i>	Up	—
<i>NCAPG</i>	Up	Up
<i>SPC25</i>	Up	Up
<i>CDCA5</i>	Up	Up
<i>CENPU</i>	Up	Up

The short horizontal line indicates that the expression of the gene does not meet the cutoff: $|\log_2FC| \geq 1.2$ and p value < 0.01 in LIHC. LIHC, liver hepatocellular carcinoma.

centromeric regions, and condensed chromosome. The downregulated DEGs were enriched for genes associated with the extracellular space, extracellular region, and extracellular region part. In the MF group, the upregulated DEGs were mainly enriched from genes associated with chromatin binding, enzyme binding, and macromolecular complex binding; and the downregulated DEGs were enriched for genes encoding proteins for cofactor binding, receptor binding, and anion binding. In the KEGG pathway group, the upregulated DEGs were enriched for genes in the cell cycle, signaling pathway, and microRNAs in cancer; and the downregulated DEGs were enriched for those associated with drug metabolism, including cytochrome P450s, chemical carcinogenesis, and the complement and coagulation cascades.

PPI networks and module analysis

All of the DEGs were uploaded onto the STRING website and were analyzed with Cytoscape software. A total of 211 nodes with 475 edges with scores > 0.900 (highest confidence) were selected to construct the PPI networks (Fig. 2). Two significant modules were selected using the MCODE plug-in. Genes in module 1 are downregulated, while the genes in module 2 are upregulated (Fig. 2B, C). Module 1

consisted of 17 nodes/genes and 99 edges (Fig. 2B), which are mainly associated with platelet degranulation (BP), secretory granule lumen (CC), receptor binding (MF), and complement and coagulation cascades (KEGG) (Table 3). Module 2 consisted of 11 nodes/genes and 49 edges (Fig. 2C), which are mainly associated with sister chromatid segregation (BP), chromosomal region (CC), histone kinase activity (MF), and the p53 signaling pathway (KEGG) (Table 4).

Validation of gene expression and overall survival of module genes

To assess whether gene expression changes are consistent between specimens from the test HCC (GSE62043) and validation LIHC (TCGA/GTEX) datasets, we validated these two module genes (total 28 genes) in the GEPIA website. We found that *CYR61*, *IGF1*, *IGF2*, and *IGFBP3* in module 1 were downregulated in tumor versus normal in the LIHC datasets (built in TCGA/GTEX), which is in accordance with GSE62043 HCC samples (Table 5). The genes of module 2, including *NDC80*, *CDK1*, *CENPF*, *CDCA8*, *CCNB1*, *BIRC5*, *NCAPG*, *SPC25*, *CDCA5*, and *CENPU*, were upregulated in tumor versus normal in the LIHC datasets, which is in accordance with GSE62043 HCC patients (Table 5).

We picked out those 14 genes with the criterion: the trend of gene expression in GSE62043 is consistent with LIHC datasets built in TCGA/GTEX. For further verification, overall survival of each of the above 14 candidate genes was calculated. As shown in Figure 3A and B, the downregulated genes (including *IGF1* and *IGF2*) have lower percent survival in the low expression group compared with the high expression group as well as the other upregulated genes (including *NDC80*, *CDK1*, *CENPF*, *CDCA8*, *CCNB1*, *BIRC5*, *NCAPG*, *SPC25*, *CDCA5*, and *CENPU*). The reason we discarded *CYR61* and *IGFBP3* is that the log-rank p value of *CYR61* did not meet standard criteria and the percent survival of *IGFBP3* is opposite to the other downregulated genes (*IGF1* and *IGF2*) (Supplementary Fig. S1). All retained candidate genes were involved in cell cycle, mitotic cell cycle, and organelle organization pathways (Fig. 4).

Discussion

This study was designed to identify potential HCC-related genes by comparing tumor tissues with adjacent nonmalignant tissues of patients suffering from HCC. Forty-eight upregulated and 377 downregulated DEGs were identified. Then, we performed GO and KEGG annotation analyses for DEGs. Subsequently, a DEG PPI network was constructed and 211 nodes/genes were identified with 475 edges, and two most significant modules were chosen from the PPIs, of which 28 central node genes were selected for further validation of gene expression and overall survival aspects in the TCGA/GTEX database. Finally, 12 genes, *IGF1*, *IGF2*, *NDC80*, *CDK1*, *CENPF*, *CDCA8*, *CCNB1*, *BIRC5*, *NCAPG*, *SPC25*, *CDCA5*, and *CENPU*, which were significantly associated with cell cycle, mitotic cell cycle, and organelle organization, were identified.

Insulin-like growth factor 1 (*IGF1*) acts in response to growth hormone. It plays an important role in childhood growth and continues to have anabolic effects in adults (Keating, 2008). Wang *et al.* (2017) demonstrated that the serum *IGF1* level is predictive of progression and survival in

FIG. 3. Overall survival of the genes with expression in accordance with that in TCGA/GTEX datasets. (A) Overall survival of downregulated genes. (B) Overall survival of upregulated genes. TCGA/GTEX, The Cancer Genome Atlas and Genotype-Tissue Expression.

FIG. 4. The functional enrichment analysis of 12 candidate hepatocellular carcinoma-related genes (gray indicates that the gene is enriched in the item, black indicates that the gene is not enriched in the item).

HCC patients. In addition to *IGF1*, the insulin-like growth factor 2 (*IGF2*) expression level is also related to the HCC stage (Adamek and Kasprzak, 2018). The nuclear division cycle 80 (*NDC80*) gene encodes one type of outer kinetochore protein. It forms a heterotetramer with the proteins *NUF2*, *SPC25*, and *SPC24*. This protein complex forms microtubule-binding domains (D'Archivio and Wickstead, 2017). Ju *et al.* (2017) showed that *NDC80* contributes to progression of HCC by reducing apoptosis and overcoming cell cycle arrest. Cyclin-dependent kinase 1 (*CDK1*) is a small protein that is highly conserved, which when coupled with *Sox2*, is closely related to tumorigenesis in human melanoma (Morgan, 2006; Ravindran Menon *et al.*, 2018). Centromere protein F (*CENPF*) is a protein encoded by the *CENPF* gene in humans (Zhu *et al.*, 1995; Chan *et al.*, 1998; Li *et al.*, 1998). Studies have shown that coexpression of *FOXM1* and *CENPF* is a robust prognostic indicator of poor survival and metastasis in human prostate cancer (Aytes *et al.*, 2014). Hayama *et al.*'s research implied that phosphorylation and activation of the cell division cycle-associated 8 (*CDCA8*) protein by aurora kinase B play an important role in human lung tumorigenesis (Satoshi *et al.*, 2007). G2/mitotic-specific cyclin-B1 (*CCNB1*), known as survivin, is a regulatory protein involved in mitosis (Sartor *et al.*, 1992). Baculoviral inhibitor of apoptosis (IAP) repeat-containing 5 (*BIRC5*) is a member of the IAP family. *BIRC5*

is involved in negative regulation of apoptosis. Survivin is known to be highly expressed in most tumor cell types and absent in normal cells, making it a good target for cancer therapy (Pennati *et al.*, 2007, 2008; Mita *et al.*, 2008; Zafarani *et al.*, 2010). Non-SMC condensin I complex subunit G (*NCAPG*) is a protein encoded by the *NCAPG* gene (Jäger *et al.*, 2000; Kimura *et al.*, 2001; Geiman *et al.*, 2004). The Liu *et al.* (2018) group suggested that silencing *NCAPG* inhibits proliferation and induces apoptosis in HCC cells. Spindle pole body component 25 homolog (*SPC25*), a component of the *NDC80* kinetochore complex, plays a crucial role in regulating mitotic chromosome segregation in prostate cancer (Liu *et al.*, 2018). Cell division cycle-associated 5 (*CDCA5*) serves an important role in promoting colorectal cancer progression by activating the ERK signaling pathway (Shen *et al.*, 2019). Additionally, *CDCA5* could be associated with HCC (Tian *et al.*, 2018), breast cancer (Phan *et al.*, 2018), and lung carcinogenesis (Nguyen *et al.*, 2010). Centromere protein U (*CENPU*) promotes nonsmall cell lung cancer proliferation involving the Wnt/ β -catenin signaling pathway and predicts poor survival through the expression of *FOXM1* (Wang *et al.*, 2018; Zhang *et al.*, 2018).

In conclusion, in this study, we have identified 12 potential candidate HCC-related genes, most of which have previously been implicated in multiple pathways associated with tumorigenesis. In addition, we identified two novel genes, *CENPU* and *SPC25*, as potential markers of HCC that have not been previously implicated in carcinogenesis. All of these DEG candidate HCC-related genes should be confirmed through molecular biological experiments.

Authors' Contributions

All authors participated in the data analysis. Bin Zhao and Zheng Wan performed the comparative analysis using bioinformatic tools. Bin Zhao interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

Author Disclosure Statement

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Supplementary Material

Supplementary Figure S1

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