Identification of potential significant genes in HBV related hepatocellular carcinoma via bioinformatical analysis

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**DOI:**  
10.21203/rs.3.rs-23897/v1

**SUBJECT AREAS**
- Cancer Biology  
- Oncology

**KEYWORDS**
- Hepatocellular carcinoma, Bioinformatical analysis, Genes, Microarray
Abstract

**Background:** The mortality rate of hepatocellular carcinoma (HCC) is the third highest worldwide. Infection with hepatitis B virus (HBV) is an important risk factor for the development of HCC. The fact that there is no available target drug for the HCC highlights the necessity to further explore its underlying mechanism.

**Methods:** Gene expression profiles of GSE121248, GSE55092 and GSE62232 were accessible from GEO database. From 129 HCC tissues and 138 normal tissues in the three profile datasets, we picked out differentially expressed genes (DEGs) using GEO2R and Venn diagram software analyzed Gene and Genome (KEGG) pathway and gene ontology (GO) in DEGs through DAVID software, and simulated the interactions between DEGs using the plotting function of STRING database, as well as constructed a protein-protein interaction (PPI) network by Cytoscape software. Consequently significant genes with potential poor prognosis were selected using UALCAN and validated in Gene Expression Profiling Interactive Analysis.

**Results:** In total of 103 DEGs in the three datasets, there were 26 up-regulated genes rich in regulation of attachment of spindle microtubules to kinetochore, protein localization to kinetochore, mitotic cytokinesis, cytokinesis, positive regulation of cytokinesis, Cell cycle and p53 signaling pathway while 77 down-regulated genes enriched in Retinol metabolism, Caffeine metabolism, Drug metabolism - cytochrome P450, Metabolism of xenobiotics by cytochrome P450, Chemical carcinogenesis, oxidation-reduction process, exogenous drug catabolic process, xenobiotic metabolic process, monocarboxylic acid metabolic process, epoxide P450 pathway and drug metabolic process. PPI network analyzed by Molecular Complex Detection (MCODE) plug-in, we found 14 hub genes including TOP2A, CCNB1, RACGAP1, DTL, PBK, NEK2, PRC1, CDK1, RRM2, BUB1B, ECT2, ANLN, HMMR, ASPM, among which demonstrated 13 genes (except PRC1) had a significantly worse prognosis based on UALCAN analysis. All of the 13 genes were highly expressed in HBV related HCC tissues compared to normal tissues through GEPIA analysis.

**Conclusion:** The significant up-regulated DEGs found by using integrated bioinformatical methods could be potential therapeutic targets for HBV related HCC patients.
1. Introduction
The mortality rate of HCC is the third highest worldwide[1]. HBV is a predominant causative agent for chronic hepatitis, cirrhosis, and HCC with approximately 257 million chronic carriers all over the world, especially in East Asia[2]. HBV regulatory protein HBx has been demonstrated to be implicated in HBV-associated oncogenesis through targeting the epigenetic control of cellular genes expression. Although, the five-year overall survival rate of HCC patients is only 50–70% [3] due to lack of effective target drug, except that surgical treatment may be effective in the early stages. Therefore, it is crucial to understand the molecular mechanisms involved in carcinogenesis and progression of HBV (+) HCC, which facilitates effective diagnosis and treatment. However, the key genes of carcinogenesis and progression of HBV (+) HCC remain largely unknown. Therefore, more reliable prognostic biomarkers should be explored as a target for improving the treatment effect and better understanding the underlying mechanism.

Bioinformatics analysis based on microarray and deep sequencing technology has been widely used to reveal molecular heterogeneity between different samples at the genomic level. And it helps us identify differentially expressed genes (DEGs) and abnormal pathways involved in the carcinogenesis and progression of HBV (+) HCC. Our investigation contributes to identifying potential key genes and therapeutic targets for the carcinogenesis and progression of HBV (+) HCC.

2. Methods

Microarray data information
In a free public database of microarray/gene profile named GEO[4] (GEO, https://www.ncbi.nlm.nih.gov/geo), we found 3 gene expression profiles of GSE121248, GSE55092 and GSE62232 containing more paired HBV (+) HCC tumor and adjacent HBV (+) liver tissues. We selected 37 normal tissues and 70 HCC tissues, 91 normal tissues and 49 HCC tissues, 10 normal tissues and 10 HCC tissues, respectively. Ethical approval was waived since this study used only publicly available data, and did not involve any experiment on animals or humans.

Data processing of DEGs
DEGs between HBV(+)HCC tumor tissue and HBV(+) liver tissue was performed in GEO2R online tool
(www.ncbi.nlm.nih.gov/geo/geo2r), with |logFC| > 2 and adjust P value < 0.05. Venn software online (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to detect common DEGs among three datasets. The DEGs with log FC < 0 was considered as down-regulated genes, while the DEGs with log FC > 0 was considered as up-regulated gene.

Gene ontology and pathway enrichment analysis

Gene ontology (GO) is a common bioinformatics tool to annotate genes and identify unique biological properties of high throughput transcriptome or genome data, which can be classified into biological process (BP), cellular component (CC), and molecular function (MF) [5]. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a useful database for realizing high level functions and utilities of biological system from large-scale molecular profiles [6]. GO function and KEGG pathway enrichment of DEGs were analyzed by the Database for Annotation, Visualization and Integrated Discovery (DAVID; version 6.8; https://david.ncifcrf.gov). Adjusted p value < 0.05 was considered statistically significant.

PPI network and module analysis

PPI information can be evaluated by an online tool, STRING (Search Tool for the Retrieval of Interacting Genes) [7]. Then, the STRING app in Cytoscape [8] was applied to examine the potential correlation between these DEGs (maximum number of interactors = 0 and confidence score ≥ 0.4). In addition, the MCODE app in Cytoscape was used to check modules of the PPI network (degree cutoff = 2, max. Depth = 100, k-core = 2, and node score cutoff = 0.2).

Survival analysis and RNA sequencing expression of core genes

UALCAN is a comprehensive, user-friendly, and interactive web resource for analyzing cancer OMICS data. It is built on PERL-CGI with high quality graphics using javascript and CSS [9]. The log rank P value and hazard ratio (HR) with 95% confidence intervals were computed and showed on the plot. To validate these DEGs, we applied the GEPIA website to analyze the data of RNA sequencing expression on the basis of thousands of samples from the GTEx projects and TCGA [10].

3. Results

3.1. Identification of DEGs in HBV (+) HCC

In 129 HBV (+) HCC tissues and 138 normal tissues in the three profile datasets, via GEO2R online
tools, we extracted 146, 390 and 515 DEGs from GSE121248, GSE55092 and GSE62232, respectively. Then, we used Venn diagram software to identify the commonly DEGs in the three datasets. Results showed that a total of 103 commonly DEGs were detected, including 77 down-regulated genes (logFC< 0) and 26 up-regulated genes (logFC> 0) in the HCC tissues (Table 1 & Fig. 1).

Table 1 All 103 commonly differentially expressed genes (DEGs) were detected from three profile datasets, including 77 down-regulated genes and 26 up-regulated genes in the HBV (+) HCC tissues compared to normal tissues.

| DEGs | Gene Name |
|------|-----------|
| Up-regulated | |
| SPINK1, CAP2, DTL, IGFBP3, CCNB1, ASPM, HMMR, AKR1B10, GPC3, ROBO1, SPP1, ZIC2, ANLN, COL15A1, PRC1, CDK1, RACGAP1, RRM2, TOP2A, PBK, SULT1C2, NEK2, ACSL4, CRNDE, BUB1B, ECT2 |
| Down-regulated | |
| CYP4A22, BBOX1, CYP26A1, CYP2A6, CNTN3, TENM1, LINC01093, CXCL14, SLC22A1, IGF1, CYP39A1, HAO2, FAM134B, MT1F, SLC25A47, MFSD2A, ZG16, HHIP, KCNN2, SLC01B3, CYP1A2, CNDO1, BCO2, FCN3, GBA3, TTC36, CLEC4G, C3P1, CYP2B6, GY2, KMO, CD5L, LPGHR, CLEC1B, MIR675, CXCL2, FOSB, LIFR, FAM65C, CLRN3, CYP2C9, LCAT, CLEC4M, VNN1, ESR1, PLAC8, HAMP, ALDOB, DNAIS1, IL3, DCN, NAT2, IL1RAP, AKR1D1, CXCL12, TMEM27, CRHBP, THRSP, IDO2, HGFAC, ADGRG7, C7, FREM2, ADH4, GPM6A, OIT3, HGF, MT1M, GLAT, CYP2B7, CYP, 2B6, GLS2, SRD5A2, ADRA1A, APOF, C9, SRPX, FCN2 |

3.2. DEGs gene ontology and KEGG pathway analysis in HBV (+) HCC

All 103 DEGs were analyzed by DAVID software and the results of GO analysis indicated that 1) for biological processes (BP), up-regulated DEGs were particularly enriched in regulation of attachment of spindle microtubules to kinetochore, protein localization to kinetochore, mitotic cytokinesis, cytokinesis, positive regulation of cytokinesis, and down-regulated DEGs in oxidation-reduction process, exogenous drug catabolic process, xenobiotic metabolic process, monooxygenase P450 pathway, and drug metabolic process; 2) for molecular function
(MF), up-regulated DEGs were enriched in protein kinase activity and down-regulated DEGs in heme binding, iron ion binding, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, oxygen binding, monooxygenase activity, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen, and arachidonic acid epoxygenase activity; 3) for GO cell component (CC), up-regulated DEGs were significantly enriched in midbody, centralspindlin complex, centrosome, cytoplasm, and cleavage furrow, and down-regulated DEGs in extracellular region, organelle membrane, and extracellular space (Table 2).

| Category       | Term                                      | Count | UniProt | Value       |
|----------------|-------------------------------------------|-------|---------|-------------|
| DOWN-regulated | oxidation-reduction process                | 13    | 4       | E-06        |
| M_BP_D         | exogenous drug catabolic process           | 4     | 1       | E-05        |
| IRECT          | xenobiotic metabolic process               | 6     | 1       | E-05        |
| M_BP_D         | monocarboxylic acid metabolic process      | 3     | 4       | E-05        |
| IRECT          | epoxygenase P450 pathway                   | 4     | 5       | E-05        |
| M_BP_D         | drug metabolic process                     | 4     | 1       | E-05        |
| IRECT          | extracellular region                       | 23    | 7       | E-05        |
| M_CC_D         | organelle membrane                         | 7     | 1       | E-05        |
| Gene Symbol | Gene Name | Intersect | Adj. P Value | P Value | Fold Change |
|-------------|-----------|-----------|--------------|---------|-------------|
| M_CC_D      | extracellular space | 13 | 0 | | |
| M_CC_D      | heme binding | 7 | 1 | | |
| M_CC_D      | iron ion binding | 7 | 2 | | |
| M_CC_D      | oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen | 5 | 6 | | |
| M_CC_D      | oxygen binding | 4 | 7 | | |
| M_CC_D      | monooxygenase activity | 4 | 0 | | |
| M_CC_D      | oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen | 3 | 0 | | |
| M_CC_D      | arachidonic acid epoxygenase activity | 3 | 0 | | |
| M_BP_D      | regulation of attachment of spindle microtubules to kinetochore | 3 | 6 | | |
| M_BP_D      | protein localization to kinetochore | 2 | 0 | | |
| GOTER            | Pathway                                | P-value |
|------------------|----------------------------------------|---------|
| M_BP_D           | mitotic cytokinesis                     | 2.00    |
| IRECT            |                                        |         |
|                  | cytokinesis                             | 2.00    |
|                  |                                        |         |
|                  | positive regulation of cytokinesis      | 2.00    |
|                  |                                        |         |
|                  | midbody                                | 5.10E-05|
|                  |                                        |         |
|                  | centralspindlin complex                 | 2.00    |
|                  |                                        |         |
|                  | centrosome                              | 4.00    |
|                  |                                        |         |
|                  | cytoplasm                               | 10.00   |
|                  |                                        |         |
|                  | cleavage furrow                         | 2.00    |
|                  |                                        |         |
|                  | protein kinase activity                 | 3.00    |

KEGG analysis results were shown in Table 3 which demonstrated that DEGs were particularly
enriched in p53 signaling pathway, Retinol metabolism, Caffeine metabolism, Drug metabolism - cytochrome P450, Metabolism of xenobiotics by cytochrome P450, Chemical carcinogenesis, Bile secretion and Metabolic pathways (P < 0.05).

Table 3 KEGG pathway analysis of differentially expressed genes in HBV (+) HCC

| Term                                         | Count | P Value   | Genes        |
|----------------------------------------------|-------|-----------|--------------|
| Retinol metabolism                           | 6     | 1.21E-04  | CYP2B6, CYP2C |
| Caffeine metabolism                          | 3     | 5.74E-04  | NAT2, CYP2A6 |
| Drug metabolism - cytochrome P450            | 5     | 0.0017768 | CYP2B6, CYP2C |
| Metabolism of xenobiotics by cytochrome P450 | 5     | 0.0024264 | CYP2B6, CYP2C |
| Chemical carcinogenesis                      | 5     | 0.0032238 | CYP2C9, ADH4  |
| Metabolic pathways                           | 18    | 0.0079574 | CNDP1, CYP2B  |
|                                             |       |           | GBA3, ADH4,   |
|                                             |       |           | F            |
| p53 signaling pathway                        | 4     | 0.0146253 | CCNB1, CDK1,  |
| Tryptophan metabolism                        | 3     | 0.0377038 | IDO2, KMO, CY |
| Cytokine-cytokine receptor interaction       | 6     | 0.0382321 | CXCL14, CXCL6 |
| Proteoglycans in cancer                      | 5     | 0.0673178 | GPC3, ESR1, IC|
| Steroid hormone biosynthesis                 | 3     | 0.0732456 | SRD5A2, CYP1  |
| Bile secretion                               | 3     | 0.0986144 | SLC01B3, KCN  |

3.3. Protein–protein interaction network (PPI) and modular analysis

A total of 103 DEGs were imported into the DEGs PPI network complex which included 96 nodes and 177 edges, including 76 down-regulated and 20 up-regulated genes (Fig. 2A). Then we applied Cytotype MCODE for further analysis and results showed that 14 central nodes which were all up-regulated genes were identified among the 96 nodes (Fig. 2B).

3.4. Analysis of core genes by the Kaplan Meier plotter and GEPIA

Kaplan Meier plotter (http://kmplot.com/analysis) was utilized to identify 14 core genes survival data. It was found that 13 genes had a significantly worse survival while 1 had no significant (P < 0.05, Table 4 & Fig. 3). Then, GEPIA was used to dig up the 13 gene expression level between cancerous
and normal people. Results reported that all genes reflected high expressed in HCC samples contrasted to normal samples (P < 0.05, Fig. 4).

**Table 4 The prognostic information of the 13 key candidate genes**

| Category                                             | Genes                                                                 |
|------------------------------------------------------|----------------------------------------------------------------------|
| Genes with significantly worse survival (P<0.05)     | TOP2A, CCNB1, RACGAP1, DTL, PBK, NEK2, CDK1, RRM2, BUB1B, ECT2, ANLN, HMMR, ASPM |
| Genes without significantly worse survival (P>0.05)   | PRC1                                                                  |

3.5. Re-analysis of 14 selected genes via KEGG pathway enrichment

To understand the possible pathway of these 13 selected DEGs, KEGG pathway enrichment was re-analyzed via DAVID (P < 0.05). Results showed that two genes (CDK1 and CCNB1) enriched in p53 signaling pathway (P = 0.0265, Table 5 & Fig. 5).

**Table 5 Re-analysis of 13 selected genes via KEGG pathway enrichment**

| Pathway ID | Name                        | Count | p-Value | Genes                  |
|------------|-----------------------------|-------|---------|------------------------|
| xtr04115   | p53 signaling pathway       | 2     | 0.0265  | CCNB1 CDK1             |
| xtr04914   | Progesterone-mediated oocyte maturation | 2     | 0.0365  | CCNB1 CDK1             |
| xtr04114   | Oocyte meiosis              | 2     | 0.0429  | CCNB1 CDK1             |

4. Discussion

HBV is a main etiologic agent of HCC, however, the molecular mechanisms underlying the development of HCC has been partially elucidated. Thus, it is particularly urgent to dig deeper underlying targeted genes and mechanisms during the development of HCC. In this article, we further analyzed and understood the mechanism of the development of HCC in patients who infected HBV through bioinformatics. However, we only selected cancerous tissues and adjacent tissues infected with HBV in HCC patients. Plus, the patient samples are not sufficient, and false positive datas are not excluded. The above conditions suggest that subsequent experiments are needed to verify the function of the targeted genes.
To understand the possible pathway of 13 selected hub genes, KEGG pathway enrichment was re-analyzed via DAVID (P < 0.05). Results showed that two genes (CDK1 and CCNB1) enriched in p53 signaling pathway.

CDK1, a member of the Ser/Thr protein kinase family, plays an essential role in the G1/S and G2/M phase transitions of eukaryotic cell cycle by interacting with CCNB1. Cyclin B1 (CCNB1), a regulatory protein, plays an important role in controlling the G2/M transition phase during mitosis. CCNB1 upregulation has been reported to be a significant prognostic marker for poor outcome in HCC [11]. Zhang Yong et al. [12] found that CCNB1 gene knockout can significantly increase the sensitivity of hepatocellular carcinoma HepG2 to the chemotherapeutic drug daunorubicin, and that the proliferation of hepatocellular carcinoma cells with low expression of CCNB1 is significantly inhibited. It can be seen that the clonal proliferation ability of HepG2 is indeed affected by the high expression of CCNB1. It is worth noting that by inhibiting the expression of CCNB1 not only effectively inhibits the ability of liver cancer cells to proliferate, but also increases their sensitivity to chemotherapy drugs. Zhang et al. [13] found that silencing CCNB1 can induce p53 reactivation and regulate apoptosis-related proteins, reduce the proliferation capacity of pancreatic cancer cells and the proportion of liver cancer cells in S phase, significantly enhance the apoptosis and aging of pancreatic cancer cells, and increase G0 / G1 phase cell ratio. Similarly, some literatures have also reported that the abnormal expression of CCNB1 can affect tumor biological effects through p53 signaling pathways [14, 15].

However, the role of these genes in HBV (+) HCC is unclear, and further research is needed. In conclusion, this study revealed that CDK1 and CCNB1 were two potential key genes for hepatocarcinogenesis, and may be candidate biomarkers and potential therapeutic targets for HBV (+) HCC.

Besides CCNB1 and CDK1, from analyzation of a PPI network with the DEGs, another hub genes, including TOP2A, RACGAP1, DTL, PBK, NEK2, PRC1, RRM2, BUB1B, ECT2, ANLN, HMMR, ASPM, were identified as the key genes in HCC.

Topoisomerase (TOP) is an important ribozyme in cells, which changes the topological structure of DNA mainly through catalysis. The role of TOP2A is to mediate the untwisting (breaking and
reconnection) of DNA double strands, affecting DNA replication, thereby initiating an apoptotic program and causing cell death. Distinct TOP2A transcriptions were confirmed in an independent series of HCC tumors relative to adjacent non-tumoral liver. By tissue microarray analysis of 172 HCC, TOP2A expressions correlated with advance histological grading, microvascular invasion and an early age onset of the malignancy [16]. Besides, Zhu et al. found the expression of TOP2A was significantly negatively correlated with the overall survival time of patients with HCC [17].

Rac GTPase active protein-1 (RacGAP1) is a member of the GTPase active protein family. Studies have found that RacGAP1 is highly expressed in gastric cancer tissues. And patients with high expression of RacGAP1 have a poor prognosis [18].

DTL (Cdc10-dependent transcript 2) is a critical regulator of cell cycle progression and genomic stability. The upregulation of DTL expression in aggressive HCC correlated positively with tumor grade and poor patient survival. DTL depletion inhibited liver cancer cell growth, increased senescence, and reduced tumorigenesis. Moreover, DTL silencing inhibited the growth of patient-derived primary cultured HCC cells [19].

Never in mitosis gene-A (NIMA)-related expressed kinase 2 (NEK2) has been recently reported to play a role in tumor progression, drug resistance and tumorigenesis. NEK2 was overexpressed in human HCC. NEK2 overexpression was significantly associated with liver noncapsulation and predicted poor survival outcomes in HCC patients after hepatectomy. In addition, NEK2 significantly enhanced HCC cell invasive ability [20].

PRC1 expression is associated with early recurrence of liver cancer and poor prognosis in patients. In HCC, PRC1 promoting tumorigenesis. And the expression and distribution of PRC1 are dynamically regulated by Wnt3a signaling [21].

Liu X et al. found that RRM2 might be targeted for HBV inhibition, and the RRM2-targeting compound osalmid and its derivative YZ51 could be a novel class of anti-HBV candidates with potential use for hepatitis B and HBV-related HCC treatment [22].

Silenced the expression of BUB1B in HepG2, a hepatocellular carcinoma cell line, found that proliferation ability and the invasion ability of hepatocellular carcinoma cells decreased. The survival
rate of HCC patients with high expression of BUB1B gene is worse than that of HCC patients with low expression of BUB1B gene. Further analysis found that among HCC patients (n = 150) with a history of hepatitis virus infection, the level of BUB1B gene expression has no effect on prognosis, only HCC patients not infected with hepatitis virus (n = 167) can be used as molecular markers to predict prognosis. BUB1B gene is highly expressed in HCC patients and promotes the proliferation and invasion of liver cancer cells [23].

The upregulation of ECT2 is significantly associated with early recurrent HCC disease and poor survival. Knockdown of ECT2 markedly suppressed Rho GTPases activities, enhanced apoptosis, attenuated oncogenicity and reduced the metastatic ability of HCC cells. Also, ECT2 is closely associated with the activation of the Rho/ERK signalling axis to promote early HCC recurrence [24].

5. Conclusion
Taken above, our bioinformatics analysis study identified two DEGs (CDK1 and CCNB1) between HCC tissues and normal tissues on the base of three different microarray datasets. Results showed that two genes could play key roles in the progression of HCC. However, these predictions should be verified by a series of experiments in the future. Anyway, these data may provide some useful information and directions into the potential bio-markers and biological mechanisms of HCC.

Abbreviations
GEO: gene expression omnibus, HCC: Hepatocellular carcinoma, GO: gene ontology, KEGG: Kyoto encyclopedia of genes and genomes, OS: overall survival, PPI: protein-protein interaction, STRING: Search Tool for the Retrieval of Interacting Genes, GEPIA: Gene Expression Profiling Interactive Analysis, TOP2A: Topoisomerase (DNA) II alpha, CCNB1: Cyclin B1, RACGAP1: Rac GTPase active protein-1, DTL: Cdc10-dependent transcript 2, NEK2: Never in mitosis gene-A (NIMA)-related expressed kinase 2, PRC1: Protein regulator of cytokinesis 1,

CDK1: Cyclin[1]dependent kinase 1, RRM2: Ribonucleotide reductase M2

Declarations
Acknowledgments
We appreciate all contributors to the GEO, UALCAN, DAVID and STRING databases for providing open-access resources for exploring cancer genomics data.
Funding
This work was supported by grant Y-2019-STD from the Foundation of Beijing Institute of Hepatology and grant 2018ZX10302205-005 from National Major Science and Technology Projects of China (CN).

Availability of data and materials
The data that support the findings of this study are available from GEO database, DAVID, STRING, and GEPIA database, as is mentioned in the “Methods” section.

Author contributions
YZ and YYG collected and analyzed the data. YZ wrote the manuscript. WYY contributed significantly to analysis and manuscript preparation; YYW, DXC, YB OY and XN L designed and supervised the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Our paper used and analyzed public datasets. So, ethical approval was not needed for this study.

Consent for publication
No conflict of interest.

Competing interests
No competing interests.

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Figures
Authentication of 103 common DEGs in the three datasets (GSE121248, GSE55092 and GSE62232) through Venn diagrams software (available online: http://bioinformatics.psb.ugent.be/webtools/Venn/). Different color meant different datasets. 

a 26 DEGs were up-regulated in the three datasets (logFC> 0). b 77 DEGs were down-regulated in three datasets (logFC < 0)
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Figure 3

Common DEGs PPI network constructed by STRING online database and Module analysis. A

There were a total of 103 DEGs in the DEGs PPI network complex. The nodes meant proteins; the edges meant the interaction of proteins; blue circles meant down-regulated DEGs and red circles meant up-regulated DEGs. B Module analysis via Cytoscape software (degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and max. Depth = 100)

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Common DEGs PPI network constructed by STRING online database and Module analysis. A

There were a total of 103 DEGs in the DEGs PPI network complex. The nodes meant proteins; the edges meant the interaction of proteins; blue circles meant down-regulated DEGs and red circles meant up-regulated DEGs. B Module analysis via Cytoscape software (degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and max. Depth = 100)
The prognostic information of the 14 core genes. Kaplan meier plotter online tools were used to identify the prognostic information of the 14 core genes and 13 of 14 genes had a significantly worse survival rate (P < 0.05).
Significantly expressed 13 genes in HBV (+) HCC patients compared to healthy people. To further identify the genes’ expression level between HBV (+) HCC and normal people, 13 genes which were related with poor prognosis were analyzed by GEPIA website. All genes had significant expression level in HCC specimen compared to normal specimen (*P < 0.05).

Red color means tumor tissues and grey color means normal tissues.
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Re-analysis of 13 selected genes by KEGG pathway enrichment. 13 high expressed genes in HCC tissues with poor prognosis were re-analyzed by KEGG pathway enrichment. Two genes (CDK1 and CCNB1) were significantly enriched in the p53 signaling pathway. Cyclin B means CCNB1, Cdc2 means CDK1.
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