Identification of hub genes and potential drugs in hepatocellular carcinoma through integrated bioinformatics analysis

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Primary research

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Abstract

Background

Hepatocellular carcinoma (HCC) is the third cancer-related cause of death in the world. Until now, the involved mechanisms during the development of HCC are largely unknown. This study aims to explore the driven-genes and potential drugs in HCC.

Methods

Three mRNA expression datasets were used to analyze the differentially expressed genes (DEGs) in HCC. The bioinformatics approaches include identification of DEGs and hub genes, GO terms analysis and KEGG enrichment analysis, construction of protein–protein interaction network. The expression levels of hub genes were validated based on TCGA, GEPIA and the Human Protein Atlas. Moreover, overall survival and disease-free survival analysis of the hub genes were further conducted by Kaplan-Meier plotter and the GEPIA. DGIdb database was performed to search the candidate drugs for HCC.

Results

Finally, 197 DEGs were identified. The PPI network was constructed using STRING software. Then ten genes were selected and considered as the hub genes. The ten genes were all closely related to the survival of HCC patients. DGIdb database predicted 39 small molecules as the possible drugs for treating HCC.

Conclusions

Our study provides some new insights into HCC pathogenesis and treatments. The candidate drugs may improve the efficiency of HCC therapy in future.

Introduction

Hepatocellular carcinoma (HCC) is one of major health problems worldwide [1, 2]. It affects more than half a million people worldwide every year, with about 30% 5-year survival rate [3, 4]. Although a variety of therapies have been used to treat HCC in the past few decades, the treatment effect is still unsatisfactory due to postoperative recurrence and drug resistance. Increasing evidence has showed that the molecular pathogenesis of HCC may be closely associated with living environment and genetic factors, such as P53 inactivation, several oncogene activation and gene mutation [5, 6]. However, the precise mechanisms underlying HCC development and progression remain unclear.

Recently, the rapid development of high-throughput RNA microarray analysis, has allowed us to better understand the underlying mechanisms and general genetic alterations involved in HCC occurrence and metastasis. RNA microarrays have been extensively applied to explore HCC carcinogenesis through gene expression profiles and identification of altered genes [7, 8, 9]. Meanwhile, many large public databases

such as The Cancer Genome Atlas (TCGA), and Gene Expression Omnibus (GEO) can be performed to screen the differentially expressed genes (DEGs) from microarray data related to the initiation and progression of HCC.

Most of HCC patients have a relatively long latent period, therefore many HCC patients are in the intermediate or advanced stage when first diagnosed, in which radical surgery is no longer desirable [10]. However, many chemotherapy are often with unsatisfactory curative effects and some severe side-effects. For an example, sorafenib shows a 3-month median survival benefit, but is related to two grade 3 drug-related adverse events namely diarrhea and hand–foot skin reaction [11]. At present, the disease-free survival (FDS) and overall survival (OS) of HCC patients remained relatively short, highlighting the importance of developing new drugs.

In the study, Three mRNA expression profiles were downloaded (GSE121248 [12], GSE64041 [13], and GSE62232 [14]) from GEO database to identify the genes correlated to HCC progression and prognosis. Integrated analysis included identifying DEGs using the GEO2R tool, overlapping three datasets using a Venn diagram tool, GO terms analysis, KEGG biological pathway enrichment analysis, PPI network construction, hub genes identification and verification, construction of hub genes interaction network, survival analysis of the screened hub genes and exploration of the candidate small molecular drugs for HCC.

Materials And Methods

Data collection

HCC and adjacent normal tissue gene expression profiles of GSE 121248, GSE64041, and GSE62232 were downloaded from GEO database (<http://www.ncbi.nlm.nih.gov/geo/>) [15]. The microarray data of GSE121248 was based on GPL571 Platforms (Affymetrix Human Genome U133 Plus 2.0 Array) and included 70 HCC tissues and 37 normal tissues (Submission date: Oct 15, 2018). The GSE64041 data was based on GPL6244 Platforms (Affymetrix Human Gene 1.0 ST Array) and included 60 biopsy pairs from HCC patients, 5 normal liver biopsies (Submission date: Dec 10, 2014). The data of GSE62232 was based on GPL571 Platforms (Affymetrix Human Genome U133 Plus 2.0 Array) and included 81 HCC cancer tissues and 10 normal liver tissues (Submission date: Oct 09, 2014). The above datasets meet the following criteria: (1) they used tissue samples from human HCC tissues and adjacent or non-tumor liver tissues; (2) each dataset involved more than 90 samples.

DEGs identification

GEO2R (<https://www.ncbi.nlm.nih.gov/geo/geo2r/>) was used to screen the DEGs in HCC tumor tissues and non-tumor liver tissues [16]. Adjusted P-values (adj. P) < 0.05 and $|\log FC| > 1$ were set as the cut-off criterion to select DEGs for every dataset microarray respectively [17]. Then, the overlapping DEGs among the three datasets was identified by Venn diagram tool (<http://bioinformatics.csic.es/tools/venny/>). Visual hierarchical cluster analysis was also performed to display the volcano plot of DEGs.

GO and KEGG pathway enrichment analysis

To explore the functions of DEGs, Enrichr database was performed to conduct GO terms analysis and KEGG pathway enrichment analysis [18]. We submitted the DEGs, including 54 upregulated genes and 143 downregulated genes, into Enrichr database. The GO terms consisted of following three parts: biological process (BP), cellular component (CC) and molecular function (MF). Adj. $P < 0.05$ was regarded as statistically significant.

Construction of PPI network and screening of hub genes

PPI networks are the networks of protein complexes due to their biochemical or electrostatic forces. The Search Tool for the Retrieval of Interacting Genes (STRING) (<https://string-db.org/cgi/input.pl/>) is a database constructed for analyzing the functional proteins association networks [19]. The screened DEGs had been submitted to the STRING database, and all PPI pairs with a combined score of > 0.4 were extracted. The degree of all nodes was calculated by Cytoscape (v3.6.1) plugin cytoHubba [20]. In the study, the genes with the top 10 highest degree values were regarded as hub genes.

Validation of hub genes

To validate the mRNA expression level of the hub genes in HCC, The GEPIA database was used to show difference in the mRNA expression level of each hub gene between liver hepatocellular carcinoma (LIHC) and non-cancerous liver samples [21]. Afterwards, the protein expression levels of the hub genes in normal and HCC tissues were visualized through the Human Protein Atlas (HPA) database that contains immunohistochemistry-based expression data for about 20 common types of cancers [22].

Genetic alterations of hub genes

The LIHC dataset (TCGA, PanCancer Atlas) including the data of 348 samples was selected for the analysis of the genetic alterations in hub genes using cBioPortal database. This database allows for visualization, analysis and downloading of a lot of cancer genomic dataset [23]. The genomic alterations included gene mutations, copy number variations, deep deletion, mRNA expression z-scores (RNA Seq V2 RSEM) with a z-score threshold of ± 2.0 and protein expression z-scores. According to the online instructions of cBioPortal, the analysis on disease-free survival (DFS) and overall survival (OS) were also carried out.

Survival analysis for hub genes

Kaplan-Meier plotter is extensively applied to explore the roles of more than 54,000 genes in OS based on 13,316 tumor samples from GEO, EGA and TCGA datasets including 364 patients with liver cancer. The relation between OS and hub genes expressed in patients with liver cancer was determined by the Kaplan-Meier survival analysis [24]. Moreover, the relation between DFS and the genes expressed in LIHC patients was explored through the online tool GEPIA database. The lower and upper 50% of gene expression were set as the standard for analysis. In the present study, HCC patients were divided into 2 groups based on

the median expression values of the hub genes. Log-rank $P < 0.01$ were regarded as statistically significant.

Drug-hub gene interaction

The screened hub genes were also regarded as the promising targets for searching drugs through the DGIdb database (<http://dgidb.genome.wustl.edu/>) [25].

This database have drug-gene interaction data from 30 disparate sources such as ChEMBL, DrugBank, Ensembl, NCBI Entrez, PharmGKB, and literature in NCBI PubMed. Drugs supported by no less than one databases or PubMed references were validated as the potential drugs. The final list only contained the drugs that have been approved by the Food and Drug Administration (FDA). Additionally, the identified target gene network was constructed through STITCH database (<http://stitch.embl.de/>), a software which also incorporated drug-gene relationships [26, 27].

Results

Identification of DEGs

According to GSE121248 dataset analysis, 943 DEGs were successfully identified, including 325 upregulated and 618 downregulated genes. For GSE64041 dataset, 289 DEGs were observed, including 87 upregulated and 202 downregulated genes. For GSE62232 dataset, 1,355 DEGs were identified, involving 817 upregulated and 538 downregulated genes. Venn analysis was performed to examine the intersection among the three DEGs profiles. Then, 197 DEGs were identified from the three profile datasets (Table 1). Obviously, 54 DEGs were significantly upregulated (Fig. 1A), while 143 DEGs were markedly downregulated (Fig. 1B) in HCC tissues. These 197 DEGs were plotted in Fig. 1C, where the red and green dots represented the upregulated and downregulated DEGs, respectively. In addition, the mRNA expression level of these 197 DEGs was visualized in the form of a heatmap using data profile GSE20347 (Additional file 1:Figure S1).

Table 1

The common DEGs of three gene expression profiles (adj. Pval. <0.05, |logFC|>1.0)

DEGs	Gene symbol
Upregulated (54)	SPINK1; TPX2; EDIL3; ASPM; FLVCR1; AKR1B10; GINS1; SRXN1; KPNA2; ANLN; NQO1; FOXM1; EZH2; CCNB2; RBM24; PRC1; CDK1; TOP2A; TXNRD1; SPARCL1; CDC6; FAM72A; MAP2; AURKA; BUB1B; DLGAP5; NMRAL1P1; LEF1; MKI67; CAP2; DTL; GPC3; CCL20; ROBO1; SPP1; SQLE; KIF20A; UBD; RRAGD; CD200; ITGA6; LCN2; MELK; SLC7A11; ITGA2; CCNA2; CDKN3; BUB1; NUF2; NCAPG; UBE2T; CENPF; NUSAP1; ECT2
Downregulated (143)	TUBE1; BBOX1; XDH; SDS; CXCL14; IGF1; DPT; CYP39A1; SLC25A47; PROZ; C8A; ZG16; MBL2; SLC10A1; SLC01B3; PRG4; CYP1A2; UROC1; FCGR2B; F9; BC02; ACSM3; CYP2C19; C3P1; LPA; CD5L; GHR; CLEC1B; TAT; LIFR; BHMT; COLEC10; VNN1; LYVE1; STEAP3; SHBG; DNASE1L3; ALDH8A1; NAT2; C7; BCHE; SAA2-SAA4; AKR1D1; CXCL12; GNMT; C1orf168; GPD1; CRHBP; EHD3; WDR72; IDO2; BDH2; CYP3A43; SLC38A4; DBH; FBP1; ADH4; OIT3; MT1M; SLC39A5; CETP; SRD5A2; ADRA1A; PBLD; SRPX; CYP4A22; KLKB1; GNAO1; ENO3; MT1G; SLC19A3; PGLYRP2; TENM1; INS-IGF2; CYP2C8; STEAP4; IL13RA2; SPP2; IGHM; MT1F; FETUB; MFSD2A; HHIP; APOA5; CYP2B7P; KCND3; PPP1R3B; LY6E; ITGA9; OLFML3; CNDP1; FCN3; GBA3; PDGFRA; CLEC4G; PHGDH; CYP2B6; CCBE1; FXYP1; PCK1; KMO; ANK3; CLRN3; MT1H; CLEC4M; NPY1R; ESR1; TDO2; VIPR1; IGFBP3; PLAC8; HAMP; DCN; IL1RAP; RDH16; CYP8B1; TMEM27; AFM; HPGD; LPAL2; THRSP; CYP4A11; STAB2; HGFAC; ADGRG7; OGDHL; PZP; SLC04C1; FREM2; BMPER; AADAT; GPM6A; HGF; MOGAT2; CYP3A4; EPHX2; GLS2; HABP2; APOF; ANGPTL1; PTGIS; GRAMD1C; SLC7A2

Figure 1 Identification of common DEGs from GSE121248, GSE64041, and GSE62232 datasets. Venn diagram of (A) upregulated and (B) downregulated DEGs based on the three GEO datasets. (C) Volcano plot of the 197 DEGs.

Red, upregulation; green, downregulation. The intersecting areas represent

the commonly altered DEGs. The t-test was used to analyze DEGs, with the

cut-off criteria of |logFC|>1.0 and adj. P < 0.05. DEGs, differentially expressed genes; GEO, Gene Expression Omnibus; logFC, log-fold change.

Functional enrichment analysis of DEGs

GO annotation and KEGG pathways enrichment analysis were conducted through Enrichr database(<http://amp.pharm.mssm.edu/Enrichr/>). Figure 2 showed the top 10 enriched GO terms and KEGG pathways. As shown in Fig. 2A, GO BP analysis revealed that these 197 DEGs were significantly enriched in epoxigenase P450 pathway, steroid metabolic process, kynurenine metabolic process and arachidonic acid metabolic process. The top four significantly enriched CC terms included condensed nuclear chromosome, centromeric region, spindle, integral component of plasma membrane, and condensed nuclear chromosome kinetochore (Fig. 2B). For GO MF analysis, the top four significantly enriched terms were oxidoreductase activity, steroid hydroxylase activity, oxidoreductase activity, heme binding (Fig. 2C). Additionally, the top four markedly enriched pathways for these 197 DEGs were Retinol metabolism, Arachidonic acid metabolism, Tryptophan metabolism and Caffeine metabolism (Fig. 2D).

Figure 2 GO annotation and KEGG pathway enrichment analysis of 197 DEGs. The top 10 enriched GO (A) BP, (B) CC, (C) MF terms and (D) KEGG

pathways. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; BP, biological process; CC, cellular component; MF, molecular function.

PPI network construction and hub genes identification

The STRING database was performed to determine the PPI network among

the 197 DEGs. The PPI network including 197 nodes (genes) and 968 edges (interactions) was constructed through STRING database (Additional file 1:Figure S2). The PPI enrichment p-value $< 1.0 \times 10^{-16}$. Ten genes with the highest degree scores were reged as the hub genes by applying the Cytoscape (v3.6.1) plugin cytoHubba. The results revealed that forkhead box M1(FOXM1) was the hub gene with the highest connectivity degree, followed by aurora kinase A (AURKA), cyclin A2 (CCNA2), cyclin-dependent kinase inhibitor 3 (CCKN3), marker of proliferation Ki-67 (MKI67),enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), cell division cycle 6 (CDC6),cyclin-dependent kinase 1 (CDK1),cyclin B1 (CCNB1),Topoisomerase (DNA) II alpha (TOP2A) (Table 2).

Using cytoHubba software,the PPI network of the screened 10 hub genes were constructed, which had a strong interaction among each other (Fig. 3A). The interaction network of 10 hub genes and their related genes was also identified by the FunRich software (Fig. 3B) [28]. The hub genes and their related genes could be enriched in many biological pathways through the enrichment functions of FunRich tool. KEGG analysis established that markedly enriched pathways for the hub genes included progesterone mediated oocyte maturation, cell cycle, cellular senescence, oocyte meiosis, p53 signaling pathway, Viral carcinogenesis, Lysine degradation, and gap junction (Fig. 3C).

Table 2
Top ten hub genes with higher degree of connectivity.

Gene symbol	Gene description	Degree
FOXM1	Forkhead box M1	36
AURKA	Aurora kinase A	34
CCNA2	Cyclin A2	34
CDKN3	Cyclin-dependent kinase inhibitor 3	34
MKI67	Marker of proliferation Ki-67	34
EZH2	Enhancer of zeste 2 polycomb repressive complex 2 subunit	33
CDC6	Cell division cycle 6	33
CDK1	Cyclin-dependent kinase 1	33
CCNB1	Cyclin B1	33
TOP2A	Topoisomerase (DNA) II alpha	33

Figure 3 Interaction network and KEGG analysis of the hub genes. (A) The top 10 hub genes in the PPI network were screened by Cytoscape (v3.6.1) plugin cytoHubba. The 10 hub genes are displayed from red (high degree value) to yellow (low degree value). (B) The PPI network of the 10 hub genes and their related genes, created by the FunRich software. (C) KEGG pathway enrichment analysis of the 10 hub genes. PPI, protein-protein interaction ; STRING, search tool for the retrieval of interacting genes; KEGG, Kyoto encyclopedia of genes and genomes.

Validation of mRNA expression levels of these 10 hub genes in HCC

First, a differential analysis on the mRNA expression levels of FOXM1, AURKA, CCNA2, CCKN3, MKI67, EZH2, CDK1, CCNB1, and TOP2A, between HCC and non-tumor liver tissues was conducted through the GEPIA database.

As showed in Fig. 4, the mRNA expression levels of (Fig. 4A) FOXM1, (Fig. 4B) AURKA, (Fig. 4C) CCNA2, (Fig. 4D) CCKN3, (Fig. 4E) MKI67, (Fig. 4F) EZH2, (Fig. 4G) CDC6, (Fig. 4H) CDK1, (Fig. 4I) CCNB1, and (Fig. 4J) TOP2A were significantly upregulated in HCC tissues ($P < 0.01$) compared to those in normal liver tissues. Hierarchical clustering analysis also performed that the mRNA expression levels of all the hub genes were significantly increased in primary hepatic cancer (TCGA Live Cancer, $n = 469$) tissues compared to non-tumor tissue samples through UCSC Xena Browser (Additional file 1:Figure S3). These findings were consistent with the obtained GEO microarray data.

Figure 4 Validation of the mRNA expression levels of (A) FOXM1, (B) AURKA, (C) CCNA2, (D) CCKN3, (E) MKI67, (F) EZH2, (G) CDC6, (H) CDK1,

(I) CCNB1, and (J) TOP2A in LIHC tissues and normal liver tissues using GEPIA database. These ten box plots are based on 369 LIHC samples (marked in red) and 160 normal samples (marked in gray). * $P < 0.01$ was considered statistically significant. LIHC, liver hepatocellular carcinoma.

Validation of protein expression levels of these 10 hub genes in HCC

The protein expression levels of these hub genes in HCC were Validated through the HPA database. Obviously, the protein expression levels of (Fig. 5A) FOXM1, (Fig. 5B) AURKA, (Fig. 5C) CCNA2, (Fig. 5D) MKI67, (Fig. 5E) EZH2, (Fig. 5G) CDK1, (Fig. 5H) CCNB1 and (Fig. 5I) TOP2A were not observed in hepatocytes of normal liver tissues, but medium or high expression levels of these hub genes were detected in HCC tissues (Fig. 5A-E and G-I). The low protein expression levels of CDC6 were detected in normal liver tissues, while medium protein expression levels of CDC6 were detected in HCC tissues (Fig. 5F). Unfortunately, the protein expression levels of CDKN3 were not explored because of pending cancer tissue analysis in HPA database. In brief, these present results showed that the mRNA and protein expression levels of the hub genes were overexpressed in HCC tissues.

Figure 5 Representative immunohistochemistry images of (A) FOXM1, (B) AURKA, (C) CCNA2, (D) MKI67, (E) EZH2, (F) CDC6, (G) CDK1, (H) CCNB1, and (I) TOP2A in HCC and non-cancerous liver tissues derived from the HPA database. HCC, hepatocellular carcinoma; HPA, Human Protein Atlas.

Frequencies of genetic alterations and prognostic values of these hub genes.

The frequencies of genetic alterations of these hub genes in LIHC were identified through the cBioPortal database. 32% of LIHC cases showed significant alterations in these 10 hub genes (Fig. 6A). The mRNA upregulation was the most important single alteration for these 10 hub genes in 78 cases (22%) of LIHC. The mRNA expression levels of these 10 hub genes in LIHC were further analyzed. The percentage change in the mRNA expression levels of FOXM1, AURKA, CCNA2, CDKN3, MKI67, EZH2, CDC6, CDK1, CCNB1, and TOP2A in LIHC were 7, 7, 2, 4, 3, 8, 8, 5, 7 and 5%, respectively (Fig. 6B). Furthermore, the relationship between the changes of these hub genes and LIHC prognosis was established through the cBioportal database. Kaplan-Meier plots were performed to compare OS, and DFS/PFS in LIHC patients with or without alterations in the mRNA expression levels of these hub genes. As showed in Fig. 8C, LIHC cases with altered hub gene showed remarkably worse OS relative to those with unaltered hub gene ($P = 5.600e-4$). LIHC cases with altered hub gene also showed significantly worse DFS ($P = 1.940e-3$) compared with those with unaltered hub gene (Fig. 6D).

Figure 6 Alteration frequency and prognosis of the 10 hub genes. (A) The summary of the cancer types was used to calculate alteration frequency of the 10 hub genes in LIHC cases. (B) mRNA expression alterations (RNA Seq V2 RSEM) of the 10 hub genes in LIHC patients. (C) OS and (D) DFS/PFS of LIHC patients with altered (red) and unaltered (blue) mRNA expression of the 10 hub genes. LIHC, liver hepatocellular carcinoma; OS, overall survival; DFS/PFS, disease-free survival/progression-free survival.

Survival analysis of the hub genes in HCC

To further explore the relationships between the 10 hub genes and HCC, OS and DFS analysis of the 10 hub genes were performed by Kaplan-Meier plotter, and the GEPIA database. As showed in Fig. 7, the high expression levels of FOXM1, AURKA, CCNA2, CDKN3, MKI67, EZH2, CDC6, CDK1, CCNB1, and TOP2A in LIHC patients were related to poor OS. The unfavorable DFS was also significantly showed in LIHC patients with high expression levels of these 10 hub genes (Fig. 8).

Figure 7 OS of the 10 hub genes overexpressed in patients with liver cancer was analyzed by Kaplan-Meier plotter. FOXM1, log-rank $P = 0.00036$; AURKA, log-rank $P = 0.0011$; CCNA2, log-rank $P = 0.00018$; CDKN3, log-rank $P = 0.0066$; MKI67, log-rank $P = 0.00011$; EZH2, log-rank $P = 6.8e-06$; CDC6, log-rank $P = 3.6e-06$; CDK1, log-rank $P = 1.1e-05$; CCNB1, log-rank $P = 3.4E-05$; and TOP2A, log-rank $P = 0.00012$. Data are presented as Log-rank P and the hazard ratio with a 95% confidence interval. Log-rank $P < 0.01$ was regarded as statistically significant. OS, overall survival.

Figure 8 DFS of LIHC patients overexpressed the 10 hub genes were analyzed by the GEPIA online database. Data are presented as log-rank P and the hazard ratio with a 95% confidence interval. FOXM1, log-rank $P = 0.00066$; AURKA, log-rank $P = 0.0012$; CCNA2, log-rank $P = 0.0037$; CDKN3, log-rank $P = 0.0074$; MKI67, log-rank $P = 4.2e-05$; EZH2, log-rank $P = 1e-04$; CDC6, log-rank $P = 0.0044$; CDK1, log-rank $P = 0.00057$; CCNB1, log-rank $P = 2.8E-06$; and TOP2A, log-rank $P = 0.00053$. Log-rank $P < 0.01$ was considered statistically significant. DFS, disease-free survival; LIHC, liver hepatocellular carcinoma.

Drug-hub gene interaction

Using the DGIdb database to explore the drug-gene interactions of the 10 hub genes, 39 drugs for possibly treating HCC were matched and determined (Additional file 1:Table S1). Promising targeted genes of these drugs include AURKB, MKI67, EZH2, TOP2A. The final list only included these drugs which were approved by FDA, and several drugs have been tested in clinical trials. Paclitaxel was considered as a potential drug for cancer therapy due to its inhibition of AURKA and TOP2A. Etoposide, an inhibitor of TOP2A, could inhibit the development of cancer through inducing DNA damaging. Using STITCH database, we constructed downstream networks of AURKA, MKI67, EZH2, and TOP2A to investigate the additional effects caused by inhibitors of these genes. Our models showed that AURKA inhibition might have possible influence on TPX2, microtubule nucleation factor (TPX2), cell division cycle 20 (CDC20), tumor protein p53 (TP53), cell division cycle 25B (CDC25B), baculoviral IAP repeat containing 5 (BIRC5); MKI67 inhibition might have possible influence on AKT serine/threonine kinase 1 (AKT1), cadherin 1 (CDH1); EZH2 inhibition might have possible influence on histone deacetylase 1 (HDAC1), BMI1 proto-oncogene, polycomb ring finger (BMI1), YY1 transcription factor (YY1), DNA methyltransferase 3 alpha (DNMT3A), DNA methyltransferase 3 beta (DNMT3B), DNA methyltransferase 1 (DNMT1), RB binding protein 4 (RBBP4), embryonic ectoderm development (EED); TOP2A inhibition might have possible influence on DNA topoisomerase I (TOP1), DNA topoisomerase II beta (TOP2B), ubiquitin C (UBC), proliferating cell nuclear antigen (PCNA), small ubiquitin-like modifier 1 (SUMO1), and SUMO2 (Additional file 1: Figures S4-7). So far, few inhibitors of AURKA, MKI67, EZH2, and TOP2A have been tested for HCC therapy. Some of these drugs were even not regarded as anti-cancer drugs (such as levofloxacin and dexrazoxane). These data could provide new insights for targeted therapy in HCC patients.

Discussion

In the present study, bioinformatics analysis was performed to identify the potential key genes and biological pathways in HCC. Through comparing the three DEGs profiles of HCC obtained from the GEO database, 54 upregulated DEGs and 143 downregulated DEGs were identified respectively (Fig. 1). Based on the degree of connectivity in PPI network, the 10 hub genes were screened and ranked, including FOXM1, AURKA, CCNA2, CDKN3, MKI67, EZH2, CDC6, CDK1, CCNB1, and TOP2A. These 10 hub genes were functioned as a group, and may play a key role in incidence and prognosis of HCC (Fig. 3A). HCC cases with high expression of the hub genes exhibited significantly worse OS and FDS compared to those with low expression of the hub genes (Figs. 7–8). Additionally, 39 identified drugs provided new insights into the targeted therapies of HCC (Supplemental file: Table S3).

Retinol metabolism, arachidonic acid metabolism, tryptophan metabolism and caffeine metabolism were most markedly enriched for HCC through KEGG pathway enrichment analysis for 197 DGEs. Metabolic alterations clearly characterize HCC tumors [29, 30]. Currently, the rapidly development of metabolomics that allows metabolite analysis in biological fluids is very useful for discovering new biomarkers. Lots of new metabolites have been identified by metabolomics approaches, and some of them could be used as biomarkers in HCC [31].

According to the degree of connectivity, the top 10 genes in PPI network were regarded as hub genes and they were validated in GEPIA database, UCSC Xena browser and HPA database. Many studies reveal that the fork-head box transcription factor FOXM1 is essential for HCC development [32, 33, 34]. Over-expression of FOXM1 has been exhibited to strongly relative to poor prognosis and progression of HCC [35, 36]. Hepatic progenitor cells of HCC have been identified in the chemical carcinogenesis model, they express cell surface markers CD44 and EpCAM [32, 37]. Interestingly, deletion of FOXM1 causes disappearance of those cells in the tumor nodules, showing that FOXM1 is critical for the CD44 positive and EpCAM positive HCC cells [32]. The hepatic cancer stem cells in human HCC lines also depend on FOXM1, because deletion of FOXM1 will lead to loss of these cancer stem cells [32]. FOXM1 is a critical downstream factor of many cancer signaling pathways, such as Wnt/ β -catenin signaling [38]. Moreover, FOXM1 stimulates expression of some multifunctional genes, like c-Myc, Oct4, Sox2 and Nanog [39, 40].

AURKA is a mitotic serine/threonine kinase that regulates cell mitosis, cell division and cell cycle progression [41]. AURKA overexpression has been observed in HCC [42]. And AURKA overexpression has been closely relative to the aggressive tumor characteristics [43], poor prognosis [44] and drug resistance [45] of HCC. AURKA was regulated by c-Myc which contributes to cancer progression in HCC [46]. Alisertib, an inhibitor of AURKA, could inhibit cell viability and induce apoptosis in HCC cells [47]. Wang et al showed genetic variations of AURKA may be a reliable biomarker for the development of HCC [48]. Our study also indicated that increased expression levels of AURKA were relative to unfavorable OS and DFS in HCC patients.

CCNA2 [49] and CCNB1 [50] are two members of the cyclin family, which regulate cell proliferation and apoptosis, and have been close related to cancer progress and patients' survival. CCNA2 [51] and CCNB1

[52, 53] have been identified in various types of tumors. CCNA2 was overexpressed in human HCC tissues [54]. Moreover, it was reported that CCNA2 was relative to a decrease in OS for HCC patients, based on the survival and expression data from TCGA [55]. Liu et al revealed that CCNB1 were highly expressed in HCC tissues compared with normal liver tissues [56]. In addition, the overexpression of CCNB1 was correlated to poor OS and DFS in HCC patients by bioinformatics analysis [57, 58]. Our study also revealed that HCC patients with a high expression level of CCNA2 or CCNB1 exhibited worse OS and DFS compared to those with a low expression level.

CDKN3 gene is involved in cell mitosis by modulating CDK1/CDK2 dephosphorylation, and its overexpression correlates with unfavorable survival in several cancers [59]. For HCC, CDKN3 not only promotes cell proliferation but also correlates with tumor pathological grade negatively [60]. CDK1, a member of the Ser/Thr protein kinase family, plays an essential role in the control of the eukaryotic cell cycle by modulating the centrosome cycle. CDK1 has been extensively investigated in ovarian cancer and colorectal cancer [61, 62]. However, little is known about the role of CDK1 in HCC carcinogenesis. A recent study has found that metformin can significantly inhibit the proliferation of HCC cells and effectively reduce the expression of CDK1 [63]. In the present study, the high expression of CDK1 associated with unfavorable OS and DFS in HCC patients.

The marker of proliferation Ki-67 expresses in all phases of the cellular cycle over than G₀ phase [64]. MKI67 protein expression in carcinomas has been intensively investigated, and the MKI67-positive cell rate has been shown to be associated with clinical-pathological features and even clinical outcome in various cancers, including HCC [65]. In a study of patients undergoing surgical resection for HCC, higher levels of MKI67 expression in tumor tissue were associated with a higher tumor grade and early tumor recurrence [66, 67]. Furthermore, staining for MKI67 and P53 are widely used to predict the clinical outcomes of HCC patients after resection and liver transplantation [68].

EZH2 is a member of the polycomb group (PcG) protein family, which modifies transcription at the epigenetic level by regulating histone and DNA methylation [69, 70]. Lots of studies have shown that many tumor suppressor genes are suppressed by EZH2 in malignancies and that EZH2 dysregulation plays a crucial role in carcinogenesis [71, 72]. In our study, the expression of EZH2 was higher in HCC tumor tissue and the high expression of EZH2 was associated with unfavorable OS and DFS in HCC patients.

CDC6 plays a critical role in the initiation of DNA replication. As cells enter the G₁ phase, CDC6 binds to the origin recognition complex (ORC) and initiates the assembly of the prereplicative complex (pre-RC) with chromatin licensing and DNA replication factor 1 (CDC1) and mini-chromosome maintenance proteins (MCMs) [73, 74]. Once phosphorylated by cyclin-dependent kinases (CDKs) at the G₁/S phase, CDC6 is released from the pre-RC and then DNA is licensed for replication. Growing evidences have suggested that deregulation of CDC6 may contribute to cancer initiation and progression [75, 76]. Overexpression of the CDC6 protein has been observed in different types of cancer [77]. Our study reveal

that the expression of CDC6 was higher in HCC tumor tissue and the high expression of CDC6 was related with unfavorable OS and DFS in HCC patients.

TOP2A, is a key nuclease that facilitates temporary cleavage and ligation cycle of DNA [78]. In all forms of topoisomerases, TOP2A is predominantly involved in proliferating cells and overexpressed in a variety of cancers (such as breast cancer, urinary bladder cancer, and ovarian carcinoma) [78]. For HCC, bioinformatics analysis showed that overexpression of TOP2A was common in HCC tumor tissues relative to those in normal liver tissues [79]. Moreover, Wong et al. found that the high expression of TOP2A was correlated with microvascular invasion, advance histological grading, chemotherapy resistance, and poor survival rate [80]. In our study, the expression of TOP2A was higher in HCC tumor tissue compared normal liver tissue, and associated with unfavorable OS and DFS in HCC patients.

A list of 39 drugs with potential therapeutic efficacy against HCC were identified through the DGIdb database. Among the 10 hub genes, the potential gene targeting the drugs are AURKB, MKI67, EZH2, and TOP2A. In Table S2, most of drugs were inhibitors of AURKB, MKI67, EZH2, and TOP2A. However, only a few of these inhibitors have been used for HCC. More studies and clinical trials were needed to identify and explore the effective drugs for HCC. Nevertheless, the present study might push new valuable insights into the individualized and targeted therapy for HCC, and the identified conventional drugs were of potentially new use.

Conclusions

In summary, the study identified commonly changed 197 DEGs in HCC through using integrated bioinformatics analysis, including 54 upregulated DEGs and 143 downregulated DEGs. And 10 hub genes (FOXM1, AURKA, CCNA2, CDKN3, MKI67, EZH2, CDC6, CDK1, CCNB1, and TOP2A) might play important roles in HCC. The expression of the hub genes was revealed to be increased in HCC, and the overexpression level predicted poor prognosis. The 10 hub genes might function as novel markers and/or targets for the early HCC detection, prognostic judgment, and targeted therapy of HCC. Additionally, a number of drugs targeting the hub genes were identified, and they could be potentially utilized for treatment of HCC patients. This study provided powerful basis for HCC studies, and further experimental studies were needed.

Abbreviations

HCC: hepatocellular carcinoma; GEO: Gene Expression Omnibus; DEGs: differentially expressed genes; GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein-protein interaction; STRING: Search Tool for the Retrieval of Interacting Genes; TCGA: The Cancer Genome Atlas.

Declarations

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Authors' contributions

Concept and design: Ping Huang; Analysis and interpretation of the data: Xiaolong Chen; acquisition of data: Xiaolong Chen and Zhixiong Xia; Making diagrams and tables of the article: Xiaolong Chen and Yafeng Wan; drafting of the article: Xiaolong Chen and Zhixiong Xia; critical revision and final approval of the article: Ping Huang.

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Availability of data and materials

The authors declare that the data supporting this study are available within the article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Supplemental Information

Additional file 1. Figure S1: The heatmap of 197 DEGs using data profile GSE64041 as a reference. **Figure S2:** PPI network was constructed by all the 197 DEGs using STRING database. **Figure S3:** Hierarchical clustering analysis of the hub genes in HCC (n=469) and normal liver tissue (n=50) was conducted using the UCSC Xena browser. **Figure S4:** Drug-hub genes network of AURKA. **Figure S5:** Drug-hub genes network of MKI67. **Figure S6:** Drug-hub genes network of EZH2. **Figure S7:** Drug-hub genes network of TOP2A. **Table S1:** Candidate drugs targeting hub genes.

Figures

Figure 1

Identification of common DEGs from GSE121248, GSE64041, and GSE62232 datasets. Venn diagram of (A) upregulated and (B) downregulated DEGs based on the three GEO datasets. (C) Volcano plot of the 197 DEGs. Red, upregulation; green, downregulation. The intersecting areas represent the commonly altered DEGs. The t-test was used to analyze DEGs, with the cut-off criteria of $|\log FC| > 1.0$ and $\text{adj. } P < 0.05$. DEGs, differentially expressed genes; GEO, Gene Expression Omnibus; logFC, log-fold change.

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Figure 2

GO annotation and KEGG pathway enrichment analysis of 197 DEGs. The top 10 enriched GO (A) BP, (B) CC, (C) MF terms and (D) KEGG pathways. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; BP, biological process; CC, cellular component; MF, molecular function.

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Figure 3

Interaction network and KEGG analysis of the hub genes. (A) The top 10 hub genes in the PPI network were screened by Cytoscape (v3.6.1) plugin cytoHubba. The 10 hub genes are displayed from red (high degree value) to yellow (low degree value). (B) The PPI network of the 10 hub genes and their related genes, created by the FunRich software. (C) KEGG pathway enrichment analysis of the 10 hub genes. PPI, protein-protein interaction ; STRING, search tool for the retrieval of interacting genes; KEGG, Kyoto encyclopedia of genes and genomes.

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Figure 4

Validation of the mRNA expression levels of (A) FOXM1, (B) AURKA, (C) CCNA2, (D) CCKN3, (E) MKI67, (F) EZH2, (G) CDC6, (H) CDK1, (I) CCNB1, and (J) TOP2A in LIHC tissues and normal liver tissues using GEPIA database. These ten box plots are based on 369 LIHC samples (marked in red) and 160 normal samples (marked in gray). * $P < 0.01$ was considered statistically significant. LIHC, liver hepatocellular carcinoma.

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Figure 5

Representative immunohistochemistry images of (A) FOXM1, (B) AURKA, (C) CCNA2, (D) MKI67, (E) EZH2, (F) CDC6, (G) CDK1, (H) CCNB1, and (I) TOP2A in HCC and non-cancerous liver tissues derived from the HPA database. HCC, hepatocellular carcinoma; HPA, Human Protein Atlas.

Figure 5

Representative immunohistochemistry images of (A) FOXM1, (B) AURKA, (C) CCNA2, (D) MKI67, (E) EZH2, (F) CDC6, (G) CDK1, (H) CCNB1, and (I) TOP2A in HCC and non-cancerous liver tissues derived from the HPA database. HCC, hepatocellular carcinoma; HPA, Human Protein Atlas.

Figure 6

Alteration frequency and prognosis of the 10 hub genes. (A) The summary of the cancer types was used to calculate alteration frequency of the 10 hub genes in LIHC cases. (B) mRNA expression alterations (RNA Seq V2 RSEM) of the 10 hub genes in LIHC patients. (C) OS and (D) DFS/PFS of LIHC patients with altered (red) and unaltered (blue) mRNA expression of the 10 hub genes. LIHC, liver hepatocellular carcinoma; OS, overall survival; DFS/PFS, disease-free survival/progression-free survival.

Figure 6

Alteration frequency and prognosis of the 10 hub genes. (A) The summary of the cancer types was used to calculate alteration frequency of the 10 hub genes in LIHC cases. (B) mRNA expression alterations (RNA Seq V2 RSEM) of the 10 hub genes in LIHC patients. (C) OS and (D) DFS/PFS of LIHC patients with altered (red) and unaltered (blue) mRNA expression of the 10 hub genes. LIHC, liver hepatocellular carcinoma; OS, overall survival; DFS/PFS, disease-free survival/progression-free survival.

Figure 7

OS of the 10 hub genes overexpressed in patients with liver cancer was analyzed by Kaplan-Meier plotter. FOXM1, log-rank P=0.00036; AURKA, log-rank P=0.0011; CCNA2, log-rank P=0.00018; CDKN3, log-rank P=0.0066; MKI67, log-rank P=0.00011; EZH2, log-rank P=6.8e-06; CDC6, log-rank P=3.6e-06; CDK1, log-rank P=1.1e-05; CCNB1, log-rank P=3.4E-05; and TOP2A, log-rank P=0.00012. Data are presented as Log-

rank P and the hazard ratio with a 95% confidence interval. Log-rank $P < 0.01$ was regarded as statistically significant. OS, overall survival.

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OS of the 10 hub genes overexpressed in patients with liver cancer was analyzed by Kaplan-Meier plotter. FOXM1, log-rank $P = 0.00036$; AURKA, log-rank $P = 0.0011$; CCNA2, log-rank $P = 0.00018$; CDKN3, log-rank $P = 0.0066$; MKI67, log-rank $P = 0.00011$; EZH2, log-rank $P = 6.8 \times 10^{-6}$; CDC6, log-rank $P = 3.6 \times 10^{-6}$; CDK1, log-

rank $P=1.1e-05$; CCNB1, log-rank $P=3.4E-05$; and TOP2A, log-rank $P=0.00012$. Data are presented as Log-rank P and the hazard ratio with a 95% confidence interval. Log-rank $P<0.01$ was regarded as statistically significant. OS, overall survival.

Figure 8

DFS of LIHC patients overexpressed the 10 hub genes were analyzed by the GEPIA online database. Data are presented as log-rank P and the hazard ratio with a 95% confidence interval. FOXM1, log-rank

P=0.00066; AURKA, log-rank P=0.0012; CCNA2, log-rank P=0.0037; CDKN3, log-rank P=0.0074; MKI67, log-rank P=4.2e-05; EZH2, log-rank P=1e-04; CDC6, log-rank P=0.0044; CDK1, log-rank P=0.00057; CCNB1, log-rank P=2.8E-06; and TOP2A, log-rank P=0.00053. Log-rank P<0.01 was considered statistically significant. DFS, disease-free survival; LIHC, liver hepatocellular carcinoma.

Figure 8

DFS of LIHC patients overexpressed the 10 hub genes were analyzed by the GEPIA online database. Data are presented as log-rank P and the hazard ratio with a 95% confidence interval. FOXM1, log-rank P=0.00066; AURKA, log-rank P=0.0012; CCNA2, log-rank P=0.0037; CDKN3, log-rank P=0.0074; MKI67, log-rank P=4.2e-05; EZH2, log-rank P=1e-04; CDC6, log-rank P=0.0044; CDK1, log-rank P=0.00057; CCNB1, log-rank P=2.8E-06; and TOP2A, log-rank P=0.00053. Log-rank P<0.01 was considered statistically significant. DFS, disease-free survival; LIHC, liver hepatocellular carcinoma.

Supplementary Files

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