Int J Clin Exp Med 2019;12(7):8123-8135
www.ijcem.com /ISSN:1940-5901/IJCEM0093235

Original Article

Screening of hepatocellular carcinoma hub genes and construction of a control network

Wenhui Niu[1], Yufeng Zhang[2], Kunying Ye[2], Guangyu Zhang[2], Qinsheng Zhang[2], Zhongqin Dang[2], Yi Zhang[1], Yating Qi[1]

[1]Henan University of Traditional Chinese Medicin, NO.156, Jin Shui Dong Road, Zheng Dong New District, Zhengzhou, Henan, People's Republic of China; [2]Department of Gastroenterology, Second Affiliated Hospital of Henan University of Traditional Chinese Medicine, Zhengzhou, Henan, People's Republic of China

Received March 2, 2019; Accepted June 5, 2019; Epub July 15, 2019; Published July 30, 2019

Abstract: Background: Hepatocellular carcinoma (HCC) is a malignant tumor that threatens human life and health. However, the molecular mechanisms of hepatocarcinogenesis remain unclear. Aim: The current study aimed to explore the molecular mechanisms of HCC through screening and functional analysis of differentially-expressed genes of HCC, providing a new method for diagnosis and treatment of HCC. Methods: Gene expression arrays of 14 pairs of HCC tissues and corresponding non-cancerous tissues were obtained from the GEO database. Next, this study identified differentially-expressed genes using the R program. The gene function and protein-protein interaction (PPI) network of DEGs was analyzed using the DAVID website and STRING database. Visualization was conducted with Cytoscape 3.7.0 software. An MCC algorithm was used to screen high connectivity hub genes. At the same time, a hub gene-miRNA regulatory network was constructed. Finally, verification of the hub genes was conducted using Kaplan-Meier and UALCAN websites. Results: A total of 285 DEGs were identified to be significantly associated with HCC tissues, including 56 upregulated and 229 downregulated genes. Gene ontology analysis revealed that downregulated genes were mainly involved in Redox and metabolic processes. Furthermore, 20 hub genes were screened. Five genes, including RRM2, CCNB1, CDK1, NCAPG, and KIF20A, were found to be highly regulated by miRNAs. Validation results showed that expression of 20 hub genes was significantly higher in HCC tissues and closely related to prognosis of HCC patients. Conclusion: Through bioinformatics analysis, the roles of DEGs in HCC were examined. Thus study may provide a new molecular target for diagnosis and treatment of HCC, providing a theoretical basis for further study of the molecular mechanisms of HCC.

Keywords: Hepatocellular carcinoma, bioinformatics, molecular mechanism, hub genes, control network

Introduction

Hepatocellular carcinoma (HCC) is a common malignant tumor type, worldwide, with extremely high morbidity and mortality rates, especially in China [1, 2]. The mortality rate of HCC ranks second among leading causes of among all cancer-associated deaths. According to statistics, approximately 600,000 patients die of HCC every year [3-6]. Although great progress has been made in diagnostic techniques, radiotherapy, interventional therapy, and surgical treatment in recent years, prognosis of HCC remains unsatisfactory, with five-year overall survival rates less than 5% [7, 8]. Moreover, liver cancer is not easily discovered. It often progresses to middle and late stages. Many patients lose the opportunity for surgery. Therefore, it is of great significance to study the molecular mechanisms of HCC, improving prevention, diagnosis, and treatment of HCC.

In recent years, with the landmark progress of gene sequencing along with the rapid spread of the internet, hundreds of biological databases have emerged rapidly. Through bioinformatics analysis, databases can be used to screen target genes, analyzing gene function conveniently and quickly. Bioinformatics provides a novel molecular target for HCC prevention, diagnosis, and treatment.

Methods

Microarray data acquisition

Expression profiles were downloaded from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). Array data for GSE84402 (Experiment type: Expression profiling by array; Platforms: GPL570, Affymetrix Human Genome U133 plus 2.0 Array; Organism: Homo sapiens) contained 14 pairs of HCC tissues and corresponding adjacent tissues. All HCC samples were extracted and analyzed from pathologically diagnosed HCC tissues and corresponding non-cancerous tissues.

Processing data and identification of DEGs

R software (version3.5.1, https://www.r-project.org/) is an excellent tool for statistical calculation and statistical mapping. It is used in the language and operating environment of statistical analysis and drawing. It can identify GEGs via the correlation R package and function. R software can be used to screen for DEGs that are differentially-expressed between hepatocellular carcinoma tissues and corresponding non-cancerous tissues. This study used R software to process and analyze raw data. First, background corrections and quantile normalization were performed on the raw data using the robust multi-array average (RMA) algorithm in R software [9]. Differentially-expressed gene (DEGs) between the tumor and normal group samples were analyzed using paired t-tests. Multiple testing was corrected by the Benjamini-Hochberg method, obtaining adjusted *P*-values [10]. Finally, genes with adjusted *P*-values < 0.05 and |log2 fold-change (log FC)| ≥ 2 indicated significance.

Gene ontology and pathway enrichment analysis of DEGs

The biological function of DEGs was predicted by Annotation, Visualization, and Integrated Discovery version 6.7 Database (DAVID, https://david.ncifcrf.gov/, accessed November 25, 2018). A comprehensive set of functional annotation tools [11], the DAVID database includes GO and KEGG analysis functions. It is an online bioinformatics analysis system that integrates biological information and analysis tools for large-scale gene and protein (gene ID, protein ID) analysis. This helps researchers access important biological information.

GO is composed of three parts: 1) cellular Components (CC), used to describe subcellular structures; 2) molecular Function (MF), used to describe gene function; and 3) biological Process (BP), used for ordered action of biomolecules, playing a broader role [12]. The Kyoto Encyclopedia of Genome and Genome (KEGG) is a database for systematic analysis of gene functions that integrates genomic, chemical, and systemic functional information. Many metabolic pathways and their relationships can be obtained by KEGG. DEGs in GSE84402 were identified by R software. They were further analyzed using DAVID online. The cut-off criteria were set at $p < 0.05$, indicating statistical significance.

Protein-protein interaction network analysis

Search Tool for the Retrieval of Interacting Genes (STRING) database (http://string-db.org/) is a database dedicated to evaluating protein-protein interactions (PPI), including direct and indirect associations [13]. Cytoscape is a graphical display, analysis, and editing software program, visualizing the network of proteins, genes, and other biomolecules [14]. Importing PPI data into Cytoscape, a protein-protein interaction network was constructed by STRING. It was visualized in Cytocape. The Cytohubba plug-in for Cytoscape was used to screen hub genes from the PPI network. Data setting conditions: Experimentally validated interactions with a combined score > 0.4 were selected as significant. Only DEGs with a degree score ≥ 20 were selected as hub genes. The top 20 genes were obtained with the highest connectivity, designated as key genes. Subsequently, the MCODE plug-in was detected using a molecular complex, using degree cutoff = 2 node score cutoff = 0.2 K-core = 2G and max. The depth = 100 truncation criterion was used to screen out the PPI module. The signal path of the fundamental factor in each module was analyzed by DAVID.

Construction of hub gene-miRNA regulatory network

Researchers downloaded miRNAs from the MiRDB database (http://mirdb.org) that might regulate the hub gene. Numerical setting: Interaction score between miRNAs and Hub genes was ≥ 80. The hub gene-miRNA regulatory network was constructed by Cytoscape software.

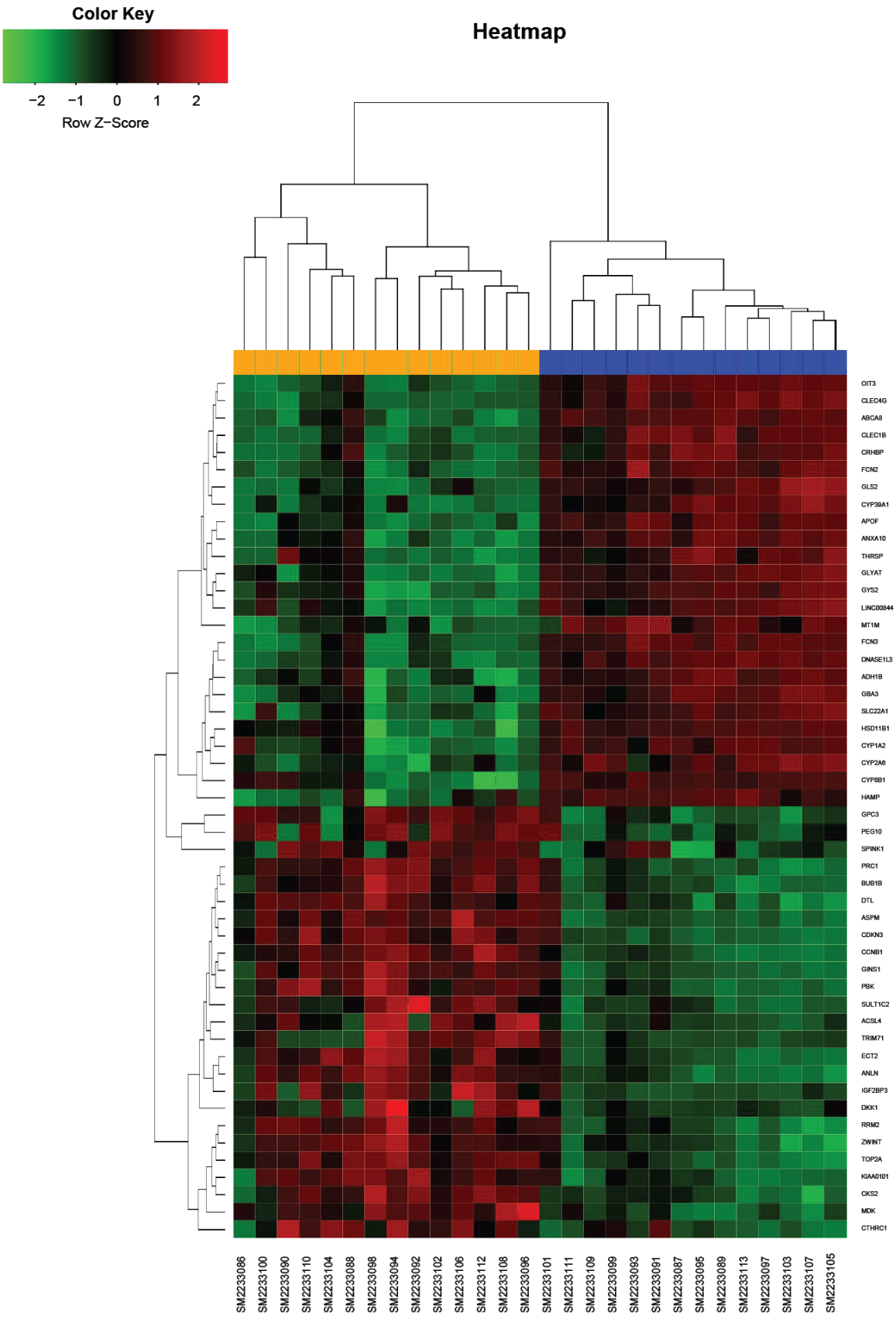

Figure 1. Heatmap of the first 25 upregulated genes and the first 25 downregulated genes. The red part represents upregulated, while the green part represents downregulated.

Table 1. Gene ontology analysis of downregulated DEGs associated with HCC

Category	ID	Term	Count	%	P	FDR[1)]
BP*	GO:0055114	Oxidation-reduction process	31	17.22	1.32E-13	2.10E-10
BP	GO:0042738	Exogenous drug catabolic process	7	3.89	7.50E-10	1.19E-06
BP	GO:0006805	Xenobiotic metabolic process	11	6.11	4.79E-09	7.63E-06
BP	GO:0019373	Epoxygenase P450 pathway	7	3.89	1.43E-08	2.28E-05
BP	GO:0071294	Cellular response to zinc ion	7	3.89	2.08E-08	3.31E-05
CC#	GO:0005576	Extracellular region	42	23.33	2.91E-09	3.42E-06
CC	GO:0070062	Extracellular exosome	56	31.11	3.95E-08	4.64E-05
CC	GO:0005615	Extracellular space	31	17.22	8.76E-06	1.03E-02
CC	GO:0031090	Organelle membrane	14	7.78	1.67E-12	1.96E-09
CC	GO:0072562	Blood microparticle	14	7.78	2.10E-09	2.47E-06
MF&	GO:0005506	Iron ion binding	14	7.78	2.92E-09	4.12E-06
MF	GO:0020037	Heme binding	13	7.22	8.58E-09	1.21E-05
MF	GO:0004497	Monooxygenase activity	11	6.11	1.87E-10	2.64E-07
MF	GO:0004252	Serine-type endopeptidase activity	10	5.56	8.45E-04	1.184607481
MF	GO:0019825	Oxygen binding	10	5.56	5.82E-10	8.20E-07

*BP biological process, #CC cell component, &MF molecular function. 1)FDR False discovery rate (E-n: ×10).

Table 2. KEGG pathway analysis of downregulated DEGs associated with HCC

Pathway ID	Term	Count	%	P[1)]	FDR[1)]
Hsa01100	Metabolic pathways	41	22.78	9.26E-07	1.11E-03
Hsa00830	Retinol metabolism	12	6.67	2.87E-09	3.45E-06
Hsa05204	Chemical carcinogenesis	11	6.11	3.60E-07	4.34E-04
Hsa00982	Drug metabolism-cytochrome P450	11	6.11	7.43E-08	8.94E-05
Hsa03320	PPAR signaling pathway	5	2.78	2.00E-02	2.16E+01

1)E-n: ×10.

UALCAN expression analysis and Kaplan-Meier survival analysis based on TCGA database

Expression and prognosis were verified by UALCAN and Kaplan-Meier plotter websites based on the TCGA database.

Results

Identification of DEGs in HCC

According to conditional analysis, 285 DEGs were identified in GSE84402. Of these, 56 were upregulated genes and 229 downregulated genes. This study compared 50 DEGs with significant differences (25 upregulated genes and 25 downregulated genes). The heat map is shown in **Figure 1**.

Gene ontology and KEGG pathway enrichment analyses

Gene functional and pathway information of the 285 DEGs were analyzed by DAVID. According to the results of GO analyses, downregulated DEGs were significantly enriched in the oxidation-reduction process, xenobiotic metabolic process, epoxygenase P450 pathways, and cellular responses to zinc ion biological processes. Cell components showed that downregulated DEGs were involved in the extracellular region, extracellular exosome, extracellular space, membrane attack complex, and integral components of the plasma membrane. Downregulated DEGs were enriched in molecular functional, including iron ion binding, heme binding, monooxygenase activity, and oxidoreductase activity (**Table 1**). Downregulated KEGG pathways were significantly enriched by metabolic pathways, retinol metabolism, chemical carcinogenesis, drug metabolism-cytochrome *P450*, and *PPAR* signaling pathways (**Table 2**). Because of the small number of upregulated genes, there were no significant enrichment results in GO and KEGG.

Module analysis of DEGs

A total of 285 DEGs (log FC > 2) were analyzed using String 10.5. Cytocape software was used

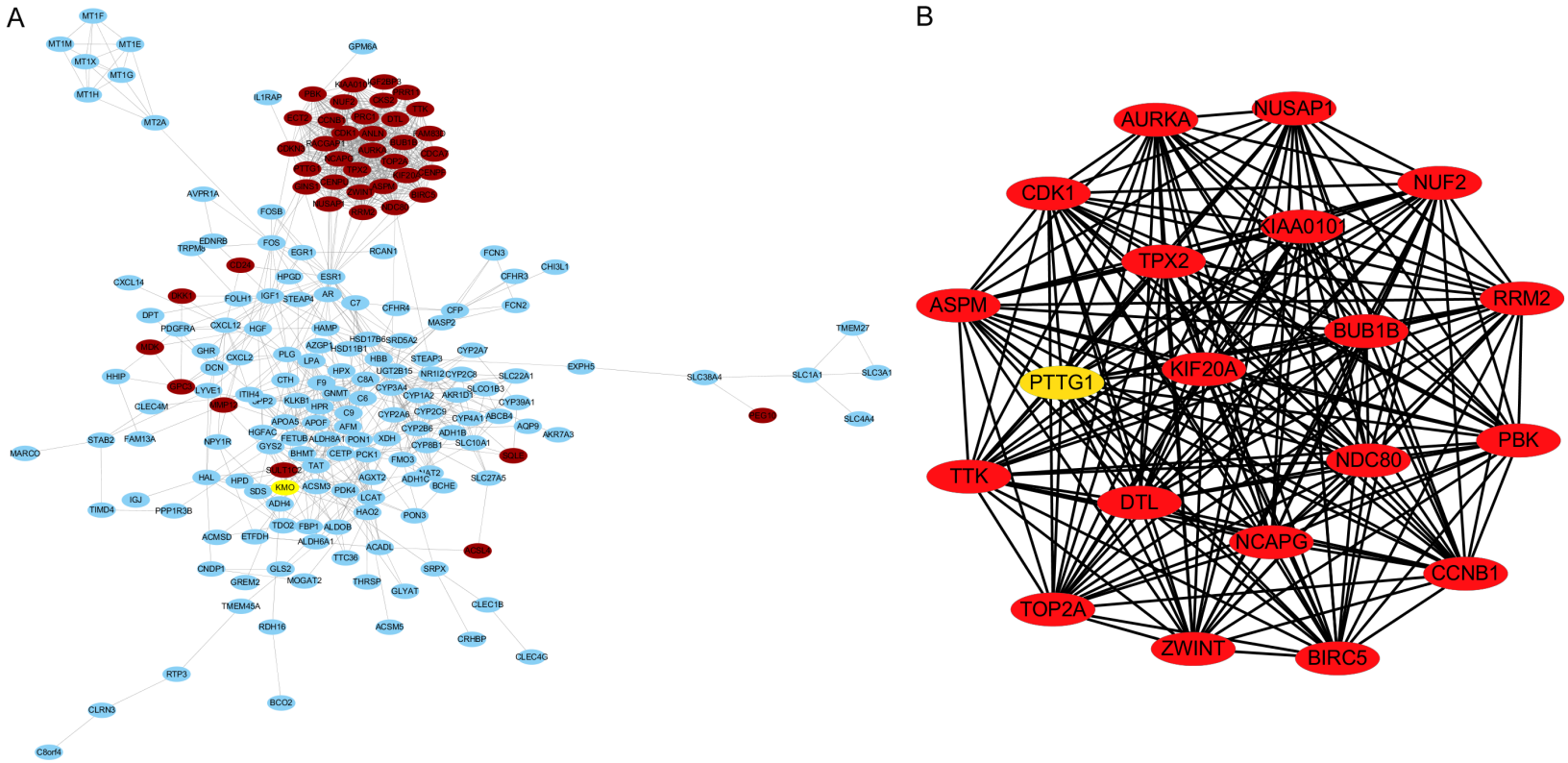

Figure 2. A. 285 DEGs were used to construct a protein-protein interaction (PPI) network by on-line analysis of String data (upregulated genes are displayed in red and downregulated genes in blue); B. Hub proteins of protein-protein interaction (PPI) network (top20).

Table 3. Twenty hub genes highly expressed in hepatocellular carcinoma tissues

Gene	Expression	Log FC	*P* Value	Adj.P.Val
BUB1B	UP	2.207781714	7.80E-08	1.63E-05
CDK1	UP	2.153406499	1.97E-08	6.68E-06
NDC80	UP	2.000320201	5.17E-08	1.27E-05
CCNB1	UP	2.890836983	3.27E-11	1.19E-07
NUF2	UP	2.038862241	4.73E-07	5.90E-05
ZWINT	UP	2.233690568	2.86E-07	4.20E-05
AURKA	UP	2.089909294	1.05E-09	1.01E-06
KIF20A	UP	2.046271049	3.57E-08	9.59E-06
NCAPG	UP	2.011597383	1.50E-06	0.000136805
TPX2	UP	2.120466798	5.16E-09	2.77E-06
BIRC5	UP	2.002120929	6.10E-09	2.98E-06
TOP2A	UP	3.206744267	8.58E-10	9.50E-07
PBK	UP	2.343555388	2.83E-08	8.41E-06
NUSAP1	UP	2.088389639	2.30E-07	3.69E-05
TTK	UP	2.177343386	1.51E-07	2.70E-05
ASPM	UP	2.923911971	9.38E-10	9.68E-07
RRM2	UP	2.556402613	3.14E-07	4.44E-05
KIAA0101	UP	2.209998721	9.27E-07	9.63E-05
DTL	UP	2.266102254	5.45E-08	1.31E-05
PTTG1	UP	2.014036076	1.71E-08	6.10E-06

to visualize the construction of the PPI network of DEGs (**Figure 2A**). Using the MCC algorithm, screening of the first 20 Hub genes and the color depth were used to represent the scores predicted by the MCC algorithm (**Figure 2B**). These 20 hub genes were highly expressed in hepatocellular carcinoma tissues (**Table 3**). Furthermore, MOCDE analysis used to group the most closely connected genes in the PPI network to form multiple modules. Results showed that the first three modules had higher scores (**Figure 3**). The most rated module 1 contained 20 Hub genes. Interactions between other genes were more dispersed.

Construction of hub gene-miRNA regulatory network

In constructing the regulatory network of the Hub gene-miRNA (**Figure 4**), a total of 72 miRNA were shown to be involved. Of these, RRM2 (connectivity = 12), CCNB1 (connectivity = 10), KIF20A (connectivity = 10), NCAPG (connectivity = 9), and CDK1 (connectivity = 6) had higher connectivity, indicating that these mRNAs were highly regulated by the miRNA network.

Expression and prognosis validation of hub genes based on TCGA database

Expression levels of hub genes were verified by the UALCAN database. Results showed that expression of Hub genes in HCC tissues was significantly higher than that in normal liver tissues. Expression levels of 10 Hub genes (**Figure 5**), including PTTG1, CCNB1, ZWINT, NUSAP1, AURKA, RRM2, CDK1, TPX2, TOP2A, and KIAA0101, were the most significant (mean > 4). This was consistent with the results of the gse84402 dataset. The survival curve of hub genes was drawn using the Kaplan-Meier survival curve method. Results showed that overall survival rates of the low expression group were better than those of the high expression group. Survival rates of the 5 hub genes, CDK1, ZWINT, PTTG1, NDC80, and AURKA, were the most significant (**Figure 6**).

Discussion

Tumorigenesis, progression, and metastasis of HCC, like most other cancers, is a complex process that is the result of a combination of multiple factors and multiple pathways, involving a large number of abnormalities in genes and pathways [15-17]. However, the molecular mechanisms of its occurrence and development have not been elucidated. To improve diagnosis and treatment of HCC, it is vitally important to find these abnormal genes or pathways, aiming to understand their roles in the molecular mechanisms of HCC. With the development of microarray and high throughput technologies, researchers have been able to detect cancer etiology by examining aberrations in whole-genome levels. These technologies have been widely used to predict potential therapeutic targets for various cancers [18].

In this study, a total of 285 differentially-expressed genes were screened by analyzing GSE84402 data of gene expression profiles in the GEO database. Differential-genes were analyzed by GO and KEGG enrichment analysis. Results showed that these DEGs were mainly involved in the biological processes of redox and other biological processes. The metabolic

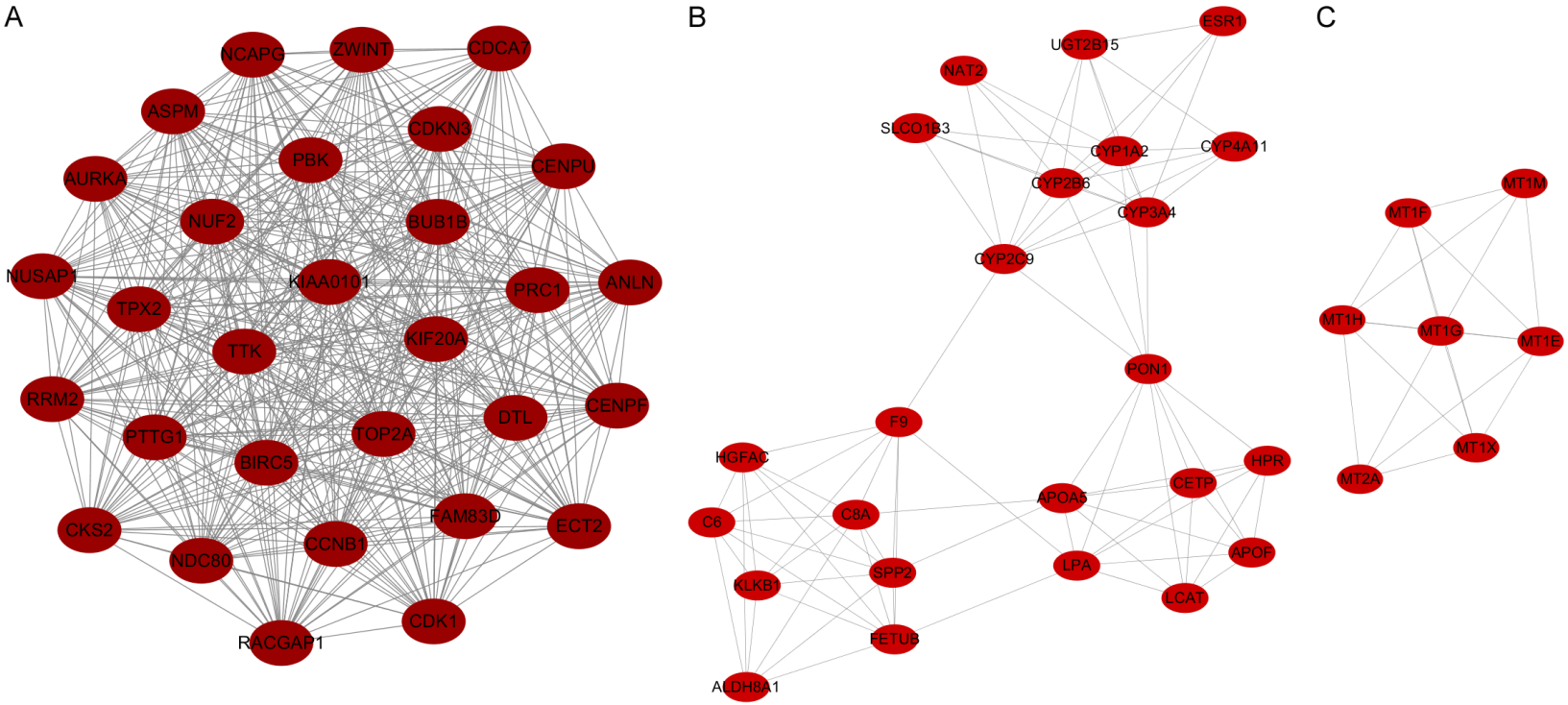

Figure 3. A. Module 1; B. Module 2; C. Module 3.

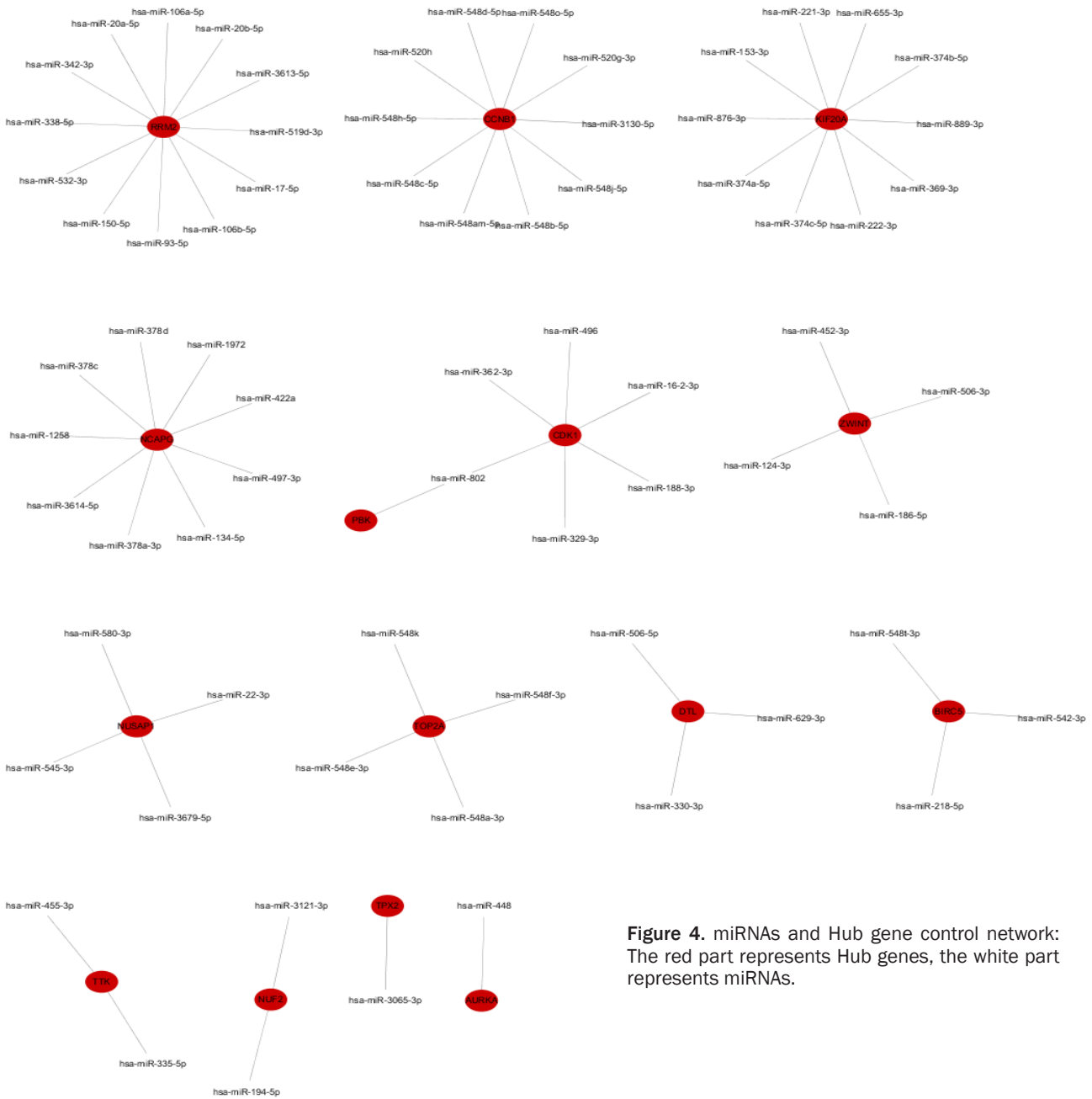

Figure 4. miRNAs and Hub gene control network: The red part represents Hub genes, the white part represents miRNAs.

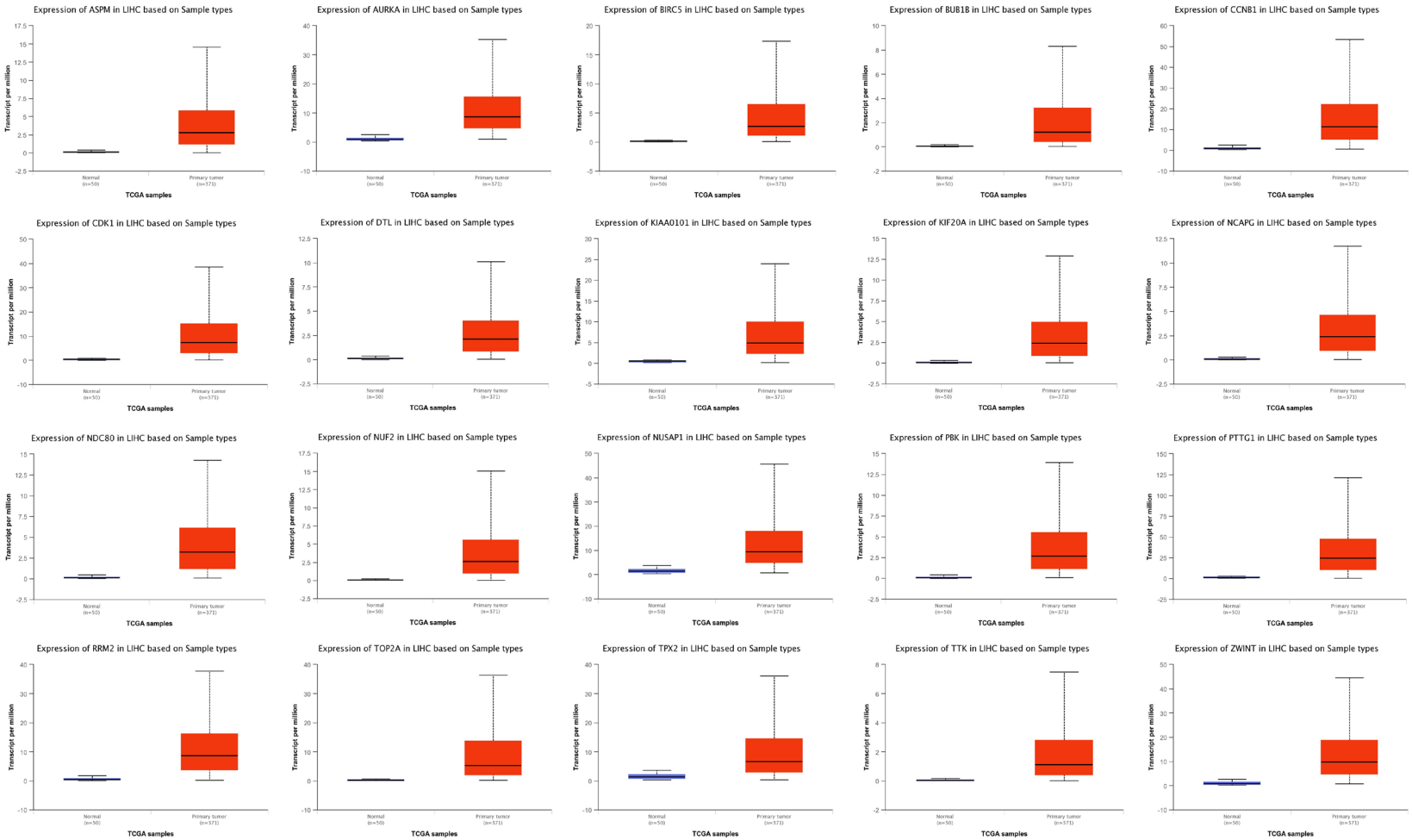

Figure 5. Verification of expression levels of hub genes. The red part represents upregulated, the blue part represents downregulated.

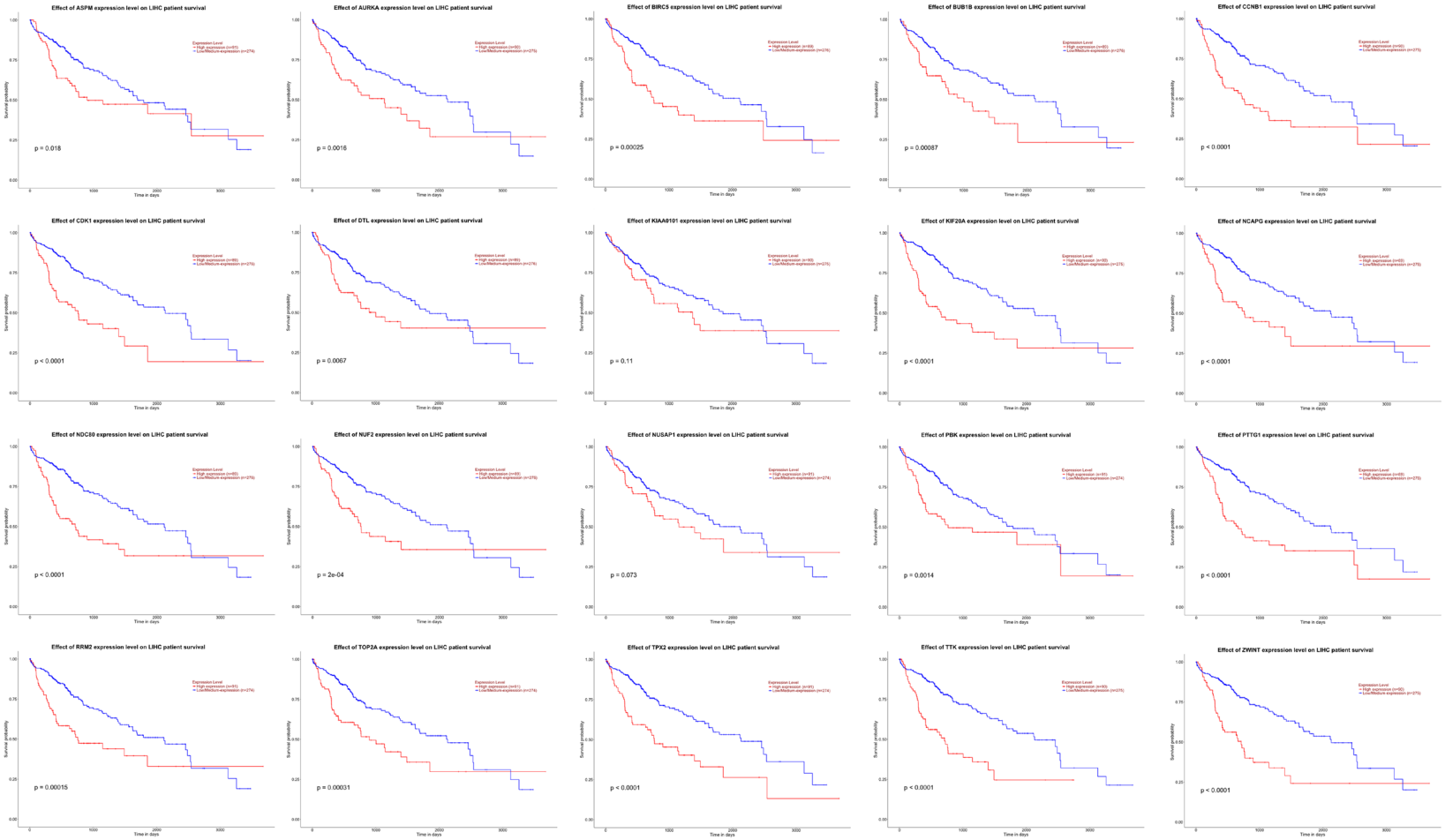

Figure 6. Kaplan-Meier survival curve analysis of Hub genes of HCC.

reprogramming of cancer cells could lead to increases in oxidative stress due to metabolism-induced changes in microenvironment. Therefore, maintaining the redox balance, cancer cells maintain redox homeostasis by enhancing their antioxidant capacity through a variety of mechanisms [19]. These genes are mainly involved in the cellular composition of extracellular bodies, organelle membranes, and membrane attack complexes. Along with glycosylation, amino acid metabolism, and other molecular functions, tumors continue to compete with the body for nutrients, especially amino acids, during the rapid growth process. Studies have shown that amino acids are an important in maintaining the growth and proliferation of tumor cells. Glutamine, leucine, and valine provide nutrients for tumor growth [20]. A total of 41 DEGs were found to be enriched in metabolic-related pathways by KEGG pathway enrichment. It also relates to PPAR signaling pathways. Peroxisome proliferate-activated receptor (PPAR) is a family of peroxisome proliferators-activated receptors, including PPAR-α, PPAR-β/δ, and PPAR-γ phenotypes. Of these, PPAR-γ plays an important role in regulating glucose metabolism and tumorigenesis and development. It has been proven that PPAR-α is highly expressed in the liver and has protective effects on liver cells [21]. In addition, PPAR-α is involved in oxidative stress and cell cycle regulation. Analysis of genes was conducted in the most closely connected and important nodes of the DEGs in the PPI network using Cytoscape software. A total of 20 Hub genes, including RRM2, CCNB1, CDK1, NCAPG, KIF20A, and other genes with the highest connectivity, were screened out. Through the construction of the hub gene-miRNA regulatory network, 70 related miRNAs were screened out. These miRNAs may have synergetic or inhibitory regulatory effects on hub genes. RRM2, CCNB1, CDK1, NCAPG, and KIF20A are highly regulated by multiple miRNAs. In subsequent verification, 20 hub genes were significantly overexpressed in HCC tissues. High expression of hub genes was shown to be closely related to poor prognosis in HCC patients.

Combining hub genes and expression and prognosis verification, the current study focused on the research progress of CDK1, PTTG1, ZWINT, NDC80, and AURKA, aiming to provide the basis for future experiments.

Research has proven that *CDK1* is involved in the regulation of cell cycle [22]. Kawamoto et al. found that *CDKI* over-proliferates in breast cancer [23]. Previous studies have suggested that *CDK1* is highly expressed in various types of cancer, including lung, colorectal, and renal cancer [24-26]. Furthermore, the activity of *CDK1* has been demonstrated to play an important role in angiogenesis, energy metabolism, and tumor development by regulation. The activity of *CDK1* has also been shown to be regulated by p53 pathways [27].

Pituitary tumor-transforming gene 1 (*PTTG1*) plays an important role in the regulation of transcription, mitosis, cell cycle regulation, chromosome stability, DNA repair, and apoptosis [28]. Many studies have shown that *PTTG1* is highly expressed in most tumors, such as thyroid cancer, colorectal cancer, ovarian cancer, thymic cancer, hepatocellular carcinoma, lung cancer, and esophageal cancer [29]. Fujii [30] reported that expression of *PTTG1* mRNA in HCC tissues was significantly higher than that in para-cancerous liver tissues and *PTTG1* protein was highly expressed in HCC tissues, but not in para-cancerous liver tissues. Moreover, high expression of *PTTG1* in HCC tissues was mainly related to the level of α-l-fucosidase, tumor size, and differentiation degree [31]. In addition, it has been proven that pituitary adenoma transforming gene 1 can promote hepatitis B virus replication and hepatitis progression by inhibiting p53 [32]. *PTTG1* gene silencing can inhibit weight gain and activity of HCC cells [33].

The protein encoded by *ZWINT* genes is the key regulation protein of mitotic checkpoint. It mainly participates in mitosis and regulates the cell cycle. It has been reported that *ZWINT* is associated with chromosomal instability (CIN), suggesting that *ZWINT* may promote tumorigenesis and development [34]. In addition, some experiments have proven that *ZWINT* protein promotes the proliferation ability of hepatoma cells by regulating cell cycle [35].

Mitotic cyclin 80 (nuclear division cycle 80 (*NDC80*) plays an important role in cell mitosis. One study showed that ndc80 induces hepatocarcinoma mainly through its effect of promoting proliferation and anti-apoptosis [36]. In addition, *NDC80* is highly expressed in osteosarcoma [37], colon cancer [38], and breast cancer [39].

AURKA is a member of the serine/threonine kinase family. It is a Ras binding protein, playing an important role in regulating cell mitosis. It has been suggested that *AURKA* may be related to MAPK signal transduction pathways mediated by Ras. This may be a new target for diagnosis and treatment of HCC [40].

Conclusion

In summary, using bioinformatics, the current study provides information on DEGs and hinge genes that may be involved in liver cancer. However, this analysis was limited. It does not capture the expression of these genes in different HCC subtypes. Another limitation of this work is that the reliability of the genes screened was not experimentally proven. Although the current work demonstrates the benefits and usefulness of microarray analysis in extracting DEGs and hub genes that could be potential diagnostic and therapeutic targets for HCC, there is a need for improved analysis and prospective clinical research. However, the current study is useful in understanding the potential molecular mechanisms of liver cancer, providing guidance for subsequent experimental studies.

Acknowledgements

Key Scientific Research projects of Colleges and Universities in Henan Province, China. (No.19A360016). Key Science and Technology Project in Henan Province, China.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yufeng Zhang, Department of Gastroenterology, Second Affiliated Hospital of Henan University of Traditional Chinese Medicine, Zhengzhou 450002, Henan, People's Republic of China. Tel: 13838071291; E-mail: jogo-2003@163.com

References

[1] Yu L, Ding GF, He C, Sun L, Jiang Y, Zhu L. MicroRNA-424 is downregulated in hepatocellular carcinoma and suppresses cell migration and invasion through c-Myb. PLoS One 2014; 9: e91661.

[2] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[3] Siegel R, Naishadham D and Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013; 63: 11-30.

[4] Stewart BW and Wild CP (eds.). World cancer report. Organization World Health, 2014. https://shop.iarc.fr/products/wcr2014.

[5] Kuo TY, Hsi E, Yang IP, Tsai PC, Wang JY, Juo SH. Computational analysis of mRNA expression profiles identifies microRNA-29a/c as predictor of colorectal cancer early recurrence. PLoS One 2012; 7: e31587.

[6] Lafaro KJ, Demirjian AN, Pawlik TM. Epidemiology of hepatocellular carcinoma. Surg Oncol Clin N Am 2015; 24: 1-17.

[7] Wallace MC, Preen D, Jeffrey GP, Adams LA. The evolving epidemiology of hepatocellular carcinoma: a global perspective. Expert Rev Gastroenterol Hepatol 2015; 9: 765-79.

[8] Yang XW, Zhang LJ, Huang XH, Chen LZ, Su Q, Zeng WT, Li W, Wang Q. miR-145 suppresses cell invasion in hepatocellular carcinoma cells: miR-145 targets ADAM17. Hepatol Res 2014; 44: 551-559.

[9] Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003; 4: 249-64.

[10] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Royal Statis Soc 1995; 57: 289-300.

[11] Gene Ontology Consortium. The gene ontology (go) project in 2006. Nucleic Acids Res 2006; 34: D322-6.

[12] Gene Ontology Consortium. Gene ontology consortium: going forward. Nucleic Acids Res 2015; 43: D1049-56.

[13] Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, Jensen LJ, von Mering C. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2017; 45: D362-D368.

[14] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software encironment for integrated models of biom olecular interaction networks. Genome Res 2003; 13: 2498-504.

[15] Vogelstein B, Kinzler KW. Cancer genes and the pathwaysthey control. Nat Med 2004; 10: 789-799.

[16] Wang WM, Xu Y, Wang YH, Sun HX, Sun YF, He YF, Zhu QF, Hu B, Zhang X, Xia JL, Qiu SJ, Zhou J, Yang XR, Fan J. HOXB7promotes tumor progression via bFGF-induced activation of MAPK/

ERKpathway and indicated poor prognosis in hepatocellular carcinoma. Oncotarget 2017; 8: 47121-47135.
[17] Kim W, Khan SK, Yang Y. Interacting network of Hippo, Wnt/β-catenin and Notch signaling represses liver tumor formation. BMB Rep 2017; 50: 1-2.
[18] Liang B, Li C and Zhao J. Identifcation of key pathways and genes in colorectal cancer using bioinformatics analysis. Med Oncol 2016; 33: 111.
[19] Chio IIC, Tuveson DA. ROS in cancer: the burning question. Trends Mol Med 2017; 23: 411-429.
[20] Locasale JW. Serine, glycine and one-carbon units: cancer metabolism in full circle. Nat Rev Cancer 2013; 13: 572-83.
[21] Juanjuan Li, Wei Du, Weilian Guo. Expression of PPAR-α and oxidative stress in normal liver tissue after TAE in hepatocellular carcinoma [J]. Guangdong Medicine 2019: 488-492.
[22] Neganova I, Tilgner K, Buskin A, Paraskevopoulou I, Atkinson SP, Peberdy D, Passos JF, Lako M. CDK1 plays an important role in the maintenance of pluripotency and genomic stability in human pluripotent stem cells. Cell Death Dis 2014; 5: e1508.
[23] Kawamoto H, Koizumi H, Uchikoshi T. Expression of the G2-M checkpoint regulators cyclin B1 and cdc2 in nonmalignant and maligant human breast lesions immunocytochemical and quantita-tive image analyses. Am J Pathol 1997; 150: 15-23.
[24] Zhang C, Elkahloun AG, Robertson M, Gills JJ, Tsurutani J, Shih JH, Fukuoka J, Hollander MC, Harris CC, Travis WD, Jen J, Dennis PA. Loss of cytoplasmic CDK1 predicts poor survival in human lung cancer and confers chemotherapeutic resistance. PLoS One 2011; 6: e23849.
[25] Sung WW, Lin YM, Wu PR, Yen HH, Lai HW, Su TC, Huang RH, Wen CK, Chen CY, Chen CJ, Yeh KT. High nuclear/cytoplasmic ratio of Cdk1 expression predicts poor prognosis in colorectal cancer patients. BMC Cancer 2014; 14: 951.
[26] Hongo F, Takaha N, Oishi M, Ueda T, Nakamura T, Naitoh Y, Naya Y, Kamoi K, Okihara K, Matsushima T, Nakayama S, Ishihara H, Sakai T, Miki T. CDK1 and CDK2 activity is a strong predictor of renal cell carcinoma recurrence. Urol Oncol 2014; 32: 1240-6.
[27] Zhai DZ, Huang QA. Research on cyclin dependent kinase cdc2 and the development of malignancy. Cancer 2006; 26: 489.
[28] Cho-Rok J, Yoo J, Jang YJ, Kim S, Chu IS, Yeom YI, Choi JY, Im DS. Adenovirus-mediated transfer of siRNA against PTTG1 inhibits liver cancer cell growth in vitro and in vivo. Hepatology 2006; 43: 1042-1052.
[29] Smith VE, Franklyn JA, Mc Cabe CJ. Pituitary tumortransforming gene and its binding factor in endocrine cancer. Expert Rev Mol Med 2010; 12: e38.
[30] Fujii T, Nomoto S, Koshikawa K, Yatabe Y, Teshigawara O, Mori T, Inoue S, Takeda S, Nakao A. Overexpression of pituitary tumor transforming gene 1 in HCC Is associated with angiogenesis and poor prognosis. Hepatology 2006; 43: 1267-75.
[31] Xuan JY, Guo YW, Zhou HX. Expression and significance of pituitary tumor transforming gene 1 in patients with hepatocellular carcinoma. Chinese Electronic Journal of Liver Surgery 2018; 7: 426-430.
[32] Peng S, Li C, Sun L. PTTG1 promotes HBV replication through P53. Journal of Microbiology 2015; 55: 935-941.
[33] Liang M, Chen X, Liu W, Li S, Li C, Jiang L, Lv S. Role of the pituitary tumor transforming gene 1 in the progression of hepatocellular carcinoma. Cancer Biol Ther 2011; 11: 337-45.
[34] Kops GJ, Weaver BA, Cleveland DW. On the road to cancer: aneuploidy and the mitotic checkpoint. Nat Rev Cancer 2005; 5: 773-85.
[35] Li Hui, Wang Genshu, Zheng Jun. Effect of high expression of zwint on proliferation of hepatoma cells and prognosis of liver transplantation. Organ Transplantation 2018; 9: 122-129.
[36] Ju LL, Chen L, Li JH, Wang YF, Lu RJ, Bian ZL, Shao JG. Effect of NDC80 in human hepatocellular carcinoma. World J Gastroenterol 2017; 23: 3675-3683.
[37] Xu B, Wu DP, Xie RT, Liu LG, Yan XB. Elevated NDC80 expression is associated with poor prognosis in osteosarcoma patients. Eur Rev Med Pharmacol Sci 2017; 21: 2045-2053.
[38] Xing XK, Wu HY, Chen HL, Feng HG. NDC80 promotes proliferation and metastasis of colon cancer cells. Genet Mol Res 2016; 15.
[39] Bièche I, Vacher S, Lallemand F, Tozlu-Kara S, Bennani H, Beuzelin M, Driouch K, Rouleau E, Lerebours F, Ripoche H, Cizeron-Clairac G, Spyratos F, Lidereau R. Expression analysis of mitotic spindle checkpoint genes in breast carcinoma: role of NDC80/HEC1 in early breast tumorigenicity, and a two-gene signature for aneuploidy. Mol Cancer 2011; 10: 23.
[40] Wan XB, Long ZJ, Yan M, Xu J, Xia LP, Liu L, Zhao Y, Huang XF, Wang XR, Zhu XF, Hong MH, Liu Q. Inhibition of aurora-a suppresses epithelial-mesenchymal transition and invasion by downregulating MAPK in nasopharyngeal carcinoma cells. Carcinogenesis 2008; 29: 1930-1937.