RESEARCH ARTICLE

Screening and Identification of Key Genes in Hepatitis B Virus-Related Hepatocellular Carcinoma Through an Integrated Bioinformatics Approach

Thanh Thi Nguyen[1*], Nga Phuong Vu[1*], Trung Sy Tran[2], Hieu Van Hoang[3], Hoai Thi-Thu Bui[1,4]

[1]Department of Tropical and Infectious Diseases, E Hospital, Hanoi, Vietnam. [2]Vietnam Military Medical University, Hanoi, Vietnam. [3]Military Institute of Traditional Medicine, Hanoi, Vietnam. [4]School of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam.

Abstract

Objective: Primary liver cancer is one of the main causes of cancer mortality globally, with hepatocellular carcinoma (HCC) being the most frequent type. Chronic hepatitis B virus (HBV) infection is leading cause of HCC. This study aimed to identify significant genes for predicting prognosis in HBV-associated HCC. **Methods:** The GSE121248 gene expression profile was obtained from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) for HBV-associated HCC were identified by analyzing this expression profile. Enrichment analyses were performed to discover the role of DEGs in biological processes, cell components, molecular functions, and pathways. Then, protein-protein interaction (PPI) was constructed and 5 hub genes were identified. Finally, survival analysis was conducted to validate the prognostic value of these genes. **Results:** A total of 20188 official gene symbols were found, and 119 DEGs were identified between HBV-associated HCC and normal liver tissues. The PPI network identified CCNB1, CDK1, TOP2A, RACGAP1, and ASPM as hub genes. Kaplan-Meier curves showed that the high expression of the hub genes had significantly lower survival. **Conclusion:** CCNB1, CDK1, TOP2A, RACGAP1, and ASPM could be potential prognostic biomarkers and therapeutic targets for HBV-associated HCC.

Keywords: Hepatitis B virus- Hepatocellular carcinoma- Bioinformatics- Hub genes- GEO

Introduction

Primary liver cancer is one of the main causes of cancer mortality globally [1], with hepatocellular carcinoma (HCC) being the most frequent type [2]. Chronic liver problems such as chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, alcohol-related liver disease, nonalcoholic fatty liver disease (NAFLD), and cirrhosis are leading triggers of HCC [3]. Over the last few decades, significant efforts have been made to establish and improve HCC prevention and monitoring for at-risk groups. Neonatal HBV vaccination has been shown to lower HBsAg seroprevalence in HBV-endemic locations and is currently recommended in the majority of nations [4, 5]. HCC surveillance in patients with cirrhosis using ultrasonography has been suggested by numerous liver disease societies [6, 7]. Currently, hepatectomy and liver transplantation are the primary surgical treatments for HCC patients, in addition, radiation or chemotherapy are frequently employed as adjuvant or palliative treatment [8]. However, conventional medical methods do not suit all individuals with HCC. As a result, a better knowledge of the underlying processes of pathophysiology and tumorigenesis for this disease at the molecular level might aid in the development of innovative early diagnostic and therapeutic therapies to enhance the management and

Corresponding Author:
Dr. Hoai Thi-Thu Bui
Department of Tropical and Infectious Diseases, E Hospital, Hanoi, Vietnam; School of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam.
Email: thuhoaihb86@gmail.com

outcome of HCC patients.

Gene profiling and signatures, which can swiftly find differentially expressed genes (DEGs), have substantially expedited cancer research during the last few decades. Several research have looked at the prognostic implications of array-based genes from HCC, however, few have found gene signatures that indicate poor prognosis for HBV-associated HCC. Thus, the aim of this study was to identify significant genes for predicting prognosis in HBV-associated HCC utilizing publicly available data and integrated bioinformatics tools.

Materials and Methods

Data collection

For this research, we chose Gene Expression Omnibus (GEO), a publicly available collection of gene/microarray profiles. This public database has been used to conduct bioinformatics research on many types of cancers, such as breast cancers [9, 10], lung cancers [11, 12], gastrointestinal cancers [13, 14], and bone cancers [15, 16].

The GSE121248 gene expression profile was obtained from the GEO database repository. This dataset used the platform GPL570 (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array for the mRNA expression profiling. A total of 70 HCC samples and 37 non-tumor tissue samples were collected from chronic HBV-associated HCC and their adjacent normal tissues in the dataset [17].

Identification of differentially expressed genes (DEGs)

First, the dataset was annotated and standardized by quantiles. Then, the limma package of the R software was used to screen the DEGs. The threshold criterion of DEGs was $|\log FC|>2$ and $p<0.05$. The Principal Components Analysis (PCA) was visualized by using the FactoMineR and factoextra packages. The M-versus-A (MA) plot, volcano plot, and heatmap of DEGs were plotted using the ggpubr and pheatmap packages.

Enrichment analysis

Functional analysis of the DEGs was conducted to further investigate the biological processes and signal pathways in which the DEGs may be engaged. Using the clusterProfiler package, Gene Ontology (GO) analysis was performed to demonstrate the dominating function of the DEGs from molecular function and biological process. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was used to determine the relationship between DEGs and signal pathways. In this enrichment analysis, all of the findings were visualized, and a statistically significant difference was defined as $P<0.05$.

Protein-protein interaction (PPI) and module analysis

The STRING platform was used to conduct PPI network analysis for differential genes [18]. The PPI of the DEGs was built using the STRING platform and analyzed using the Cytoscape program. Cytoscape is an open-source bioinformatics application for visualizing gene and protein molecular interaction networks. Moreover, the Cytoscape plug-in Cytohubba was used to identify the hub genes that are strongly associated with HBV-associated HCC [19].

Survival Analysis

The hub genes' prognostic value was evaluated using the GEPIA database. GEPIA, an interactive web server, can perform interactive and configurable tasks such as differential expression analysis, profile charting, correlation analysis, patient survival analysis, related gene recognition, and dimensionality reduction analysis [20].

Results

Identification of DEGs

A total of 20188 official gene symbols were found, and each gene's expression was determined. 119 DEGs were identified between HBV-associated HCC and normal liver tissues using the established criteria, comprising 30 up-regulated genes and 89 down-regulated genes. Figure 1 depicts the volcano map of all genes. The top 5 up-regulated genes are CAP2, TOP2A, RACGAP1, ASPM and COL15A1, the top 5 down-regulated genes are CXCL14, IGFALS, CLEC1B, HHIP and CDHR2. Figure 2 shows the cluster heatmap of the DEGs.

GO and KEGG pathway enrichment analysis

To do GO functional annotation and KEGG pathway enrichment analysis, we utilized the clusterProfiler R package. Figure 3 illustrates the findings of the substantial enrichment analysis. GO analysis results showed that changes in the biological process of DEGs were significantly enriched in regulation of hormone levels, response to xenobiotic stimulus, steroid metabolic process, and cellular hormone metabolic process (Figure 3A). Changes in cell component were mainly enriched in collagen-containing extracellular matrix, blood microparticle, midbody, collagen trimer, and immunoglobulin complex (Figure 3B). Figure 3C showed the changes in molecular function of DEGs, which were mainly enriched in oxidoreductase activity, monooxygenase activity, iron ion binding, heme binding, and tetrapyrrole binding. The results of KEGG pathway analysis (Figure 3D) revealed that DEGs were mostly enriched in retinol metabolism, drug metabolism - cytochrome P450, p53 signaling pathway, metabolism of xenobiotics by cytochrome P450, and steroid hormone biosynthesis.

Construction of PPI Network and identifying of hub genes

The STRING database generated a PPI network with 79 nodes and 212 edges. The PPI was then displayed in Cytoscape to investigate the functional relationships between DEGs (Figure 4A). Following that, as demonstrated in Figure 4B, the top five hub genes found in the Cytohubba plug-in using the Maximal Clique Centrality algorithm were CCNB1, CDK1, TOP2A, RACGAP1, and ASPM.
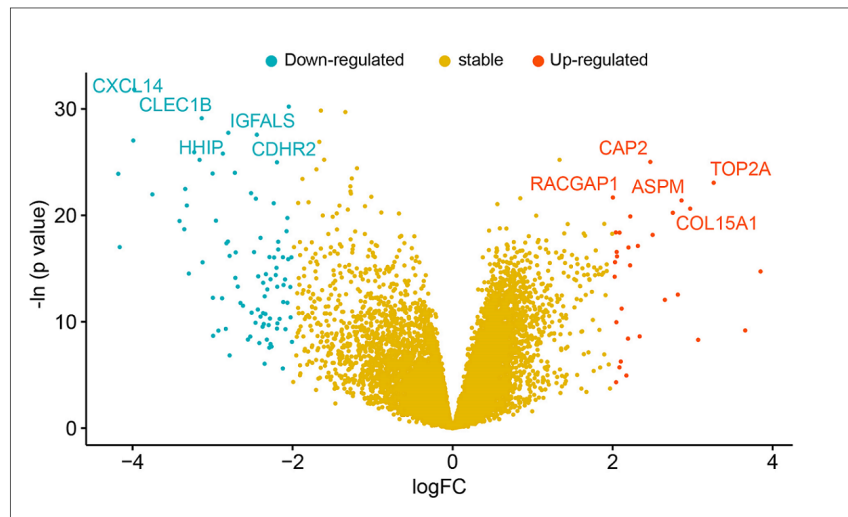
Figure 1. Volcano Plot of the Differentially Expressed Genes (DEGs). Significantly up-regulated genes, significantly down-regulated genes and non-DEGs are displayed as red, blue, and yellow dots, respectively, in HBV-associated HCC and their adjacent normal tissues. The top ten upregulated and downregulated DEGs are highlighted.

Validation of prognostic value of the hub genes

The GEPIA online tool was used to assess the predictive significance of the identified hub genes specific to HBV-associated HCC patients. Based on the medium value of each gene, the patients were divided into the high expression group and low expression group. The Kaplan-Meier curve was used to depict the overall survival analysis between groups. Figure 5 shows that the high expression group of each gene had significantly lower survival than the low expression group (all P<0.05).

Discussion

Despite significant advances in clinical management and pathophysiology prediction for HCC in past few years, the high rate of HCC-specific death remains a significant problem [21]. Chronic HBV infection serves as the most common cause of HCC. In endemic locations, particularly in underdeveloped nations, HBV infection is mostly transmitted vertically. As a result, the typical age of HBV carriers developing HCC is younger than for other
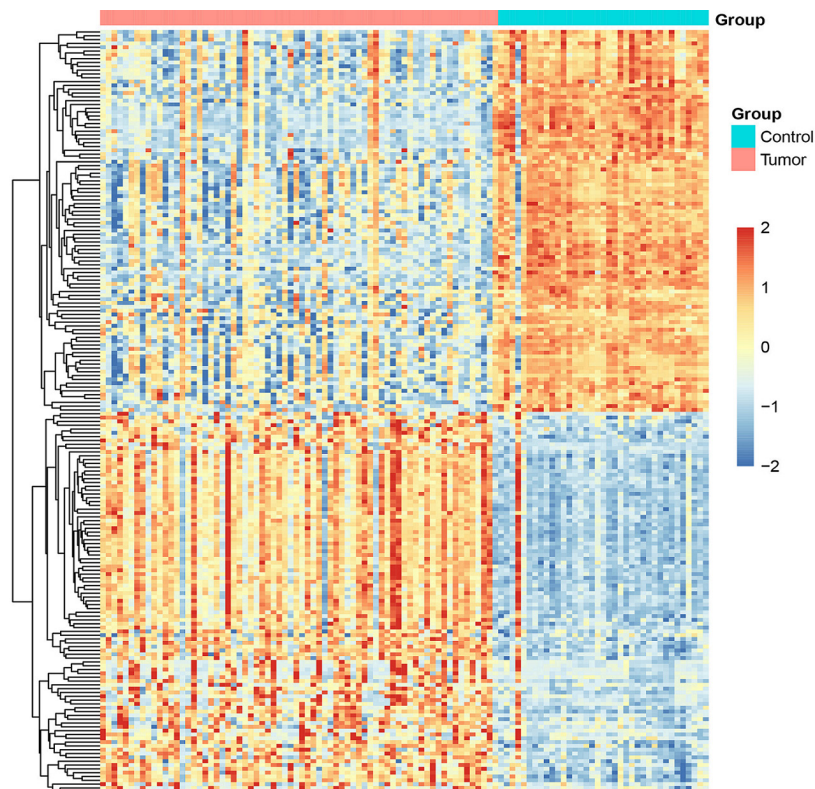

Figure 2. Cluster Heatmap of the Differentially Expressed Genes (DEGs).
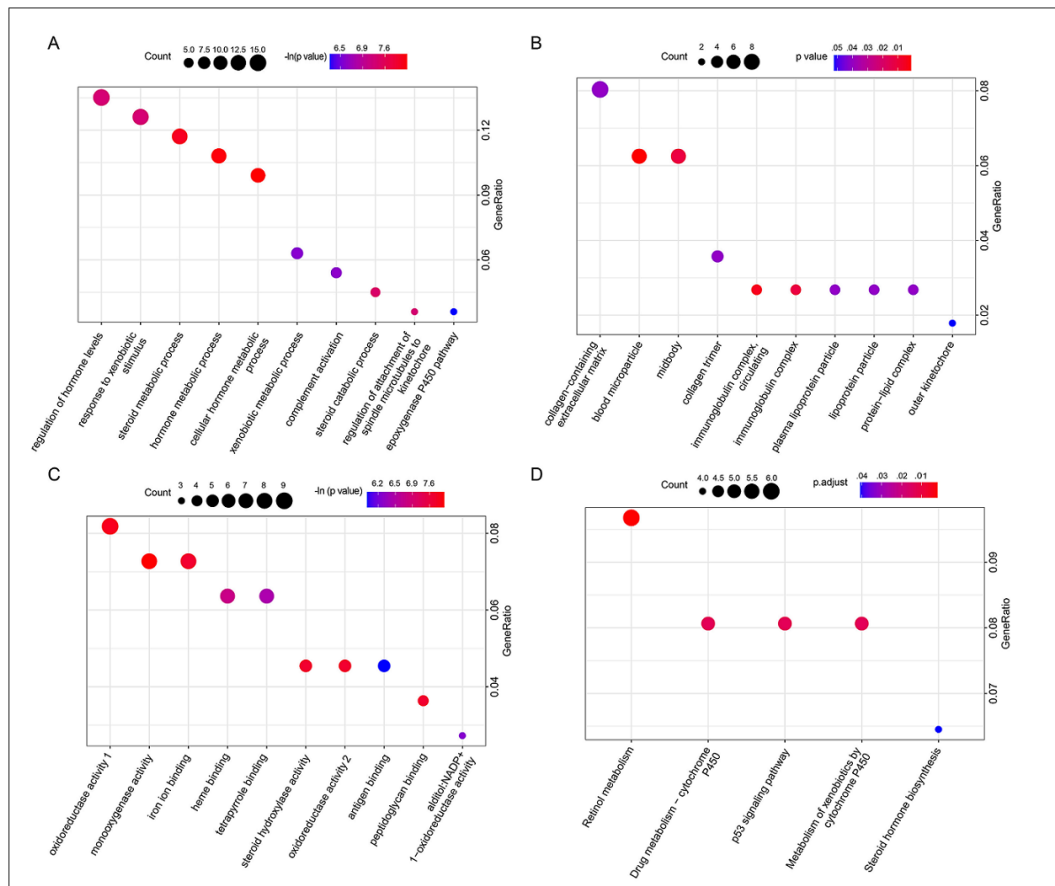
Figure 3. Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis of Differentially Expressed Genes (DEGs). A, Biological Process analysis; B, Cellular Component analysis; C, Molecular Function analysis; D, KEGG pathway analysis.

etiologies. Furthermore, the majority of patients acquire liver fibrosis and cirrhosis as a result of immunological responses during HBV infection, which promotes the development of HCC. We hoped to get new insights into the molecular mechanisms underpinning HBV-associated HCC formation and progression by using bioinformatics analyses.

This research discovered 119 DEGs including 30 up-regulated genes and 89 down-regulated genes, these DEGs are involved in retinol metabolism, drug metabolism - cytochrome P450, p53 signaling pathway, xenobiotic metabolism via cytochrome P450, and steroid hormone biosynthesis. Furthermore, five hub genes (CCNB1, CDK1, TOP2A, RACGAP1, and ASPM) were verified and found to be related to poorer outcomes in HCC populations with HBV infection.

CCNB1 encoding cyclin B1, a regulatory protein that is important in the mitosis process, is a key member of the conserved cyclin B family [22]. The abnormal activity of this gene can disorganize the cell cycle and cell proliferation, resulting in triggering oncogenesis and cancer development [23, 24]. CCNB1 was shown that substantially related to development, proliferation, migration, and invasion in HCC tumors [25]. Peng et al. [26] indicated that the miR-16/cyclin-B1 axis is the target of zingiberene in the regulation of the growth and invasion of liver tumor cells. Another study also

suggests that cyclin B1 may possibly become a biomarker and therapeutic target for HBV-associated HCC [27].

CDK1 is a serine-threonine protein kinase encoding cyclin dependent kinase 1. The abnormal expression of cyclin dependent kinase 1 strongly correlates with carcinogenesis due to its important role in cell mitosis. The antimalarial medicine dihydroartemisinin suppresses the growth of liver cancer cells by lowering CDK1 and CCNB1 expression levels [28]. Wu et al. [29] demonstrated that anti-CDK1 therapy can improve sorafenib anti-tumor response in HCC patient-derived xenograft tumor models by inhibiting CDK1/PDK1/β-Catenin signaling.

TOP2A encodes DNA topoisomerase II alpha, an enzyme that regulates and modifies the topologic states of DNA during transcription. This gene is a target for various anticancer drugs, and a number of mutations in this gene have been linked to the development of treatment resistance [30-32]. TOP2A expression level was shown to be higher in HBV-associated HCC tissues and resulting in a worse prognosis. The findings were consistent with earlier research. Furthermore, the expression of TOP2A in HCC tumors seemed to be associated with the presence of serum HbsAg [33], this might explain why TOP2A was one of the hub genes in HBV-associated HCC.

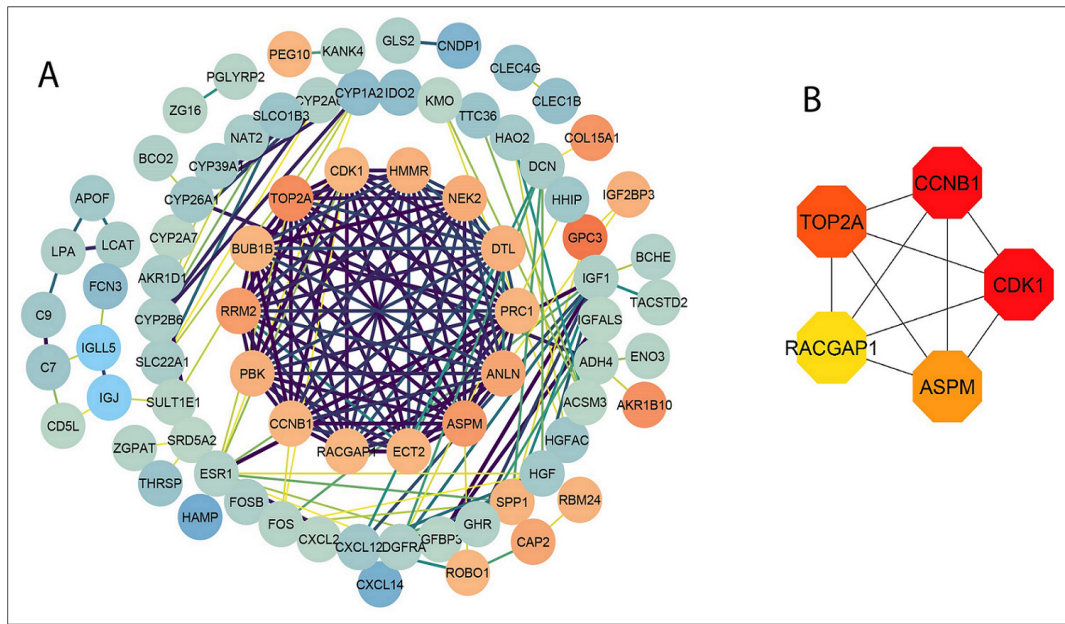RACGAP1 encodes a GTPase-activating protein that is part of the centralspindlin complex that regulates

Figure 4. Network of Protein-Protein Interaction (PPI). A. PPI network of all DEGs; B. Top 5 hub genes identified using the cytoHubba plug-in.
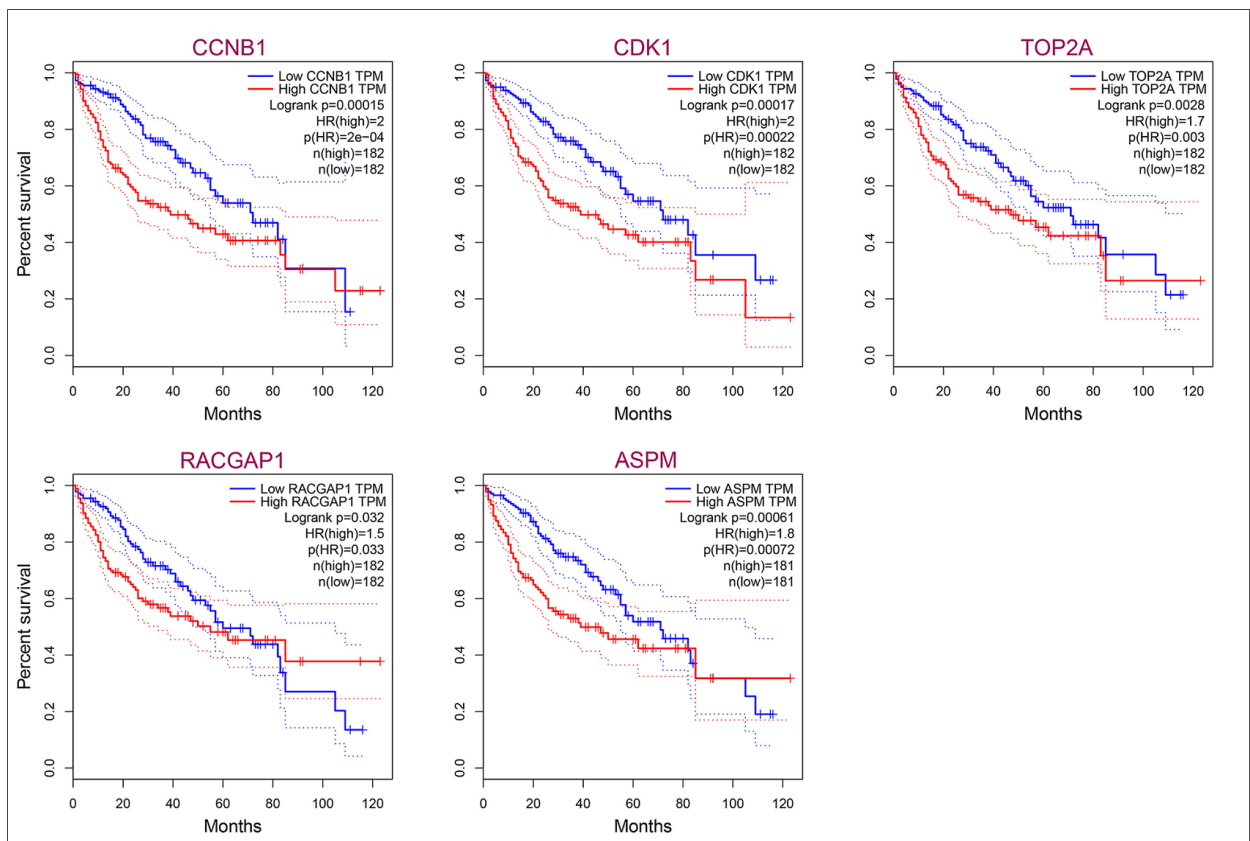

Figure 5. Kaplan-Meier Analysis for Overall Survival between High Expression Group and Low Expression Group in Hepatocellular Carcinoma.

cytokinesis, cell proliferation, and differentiation. RACGAP1 has been found as a prognostic biomarker for early HCC identification as well as a therapeutic target for liver cancer and liver cancer stem cells [34]. Wang et al. [35] discovered that the pseudogene RACGAP1P acts as a ceRNA to promote the RACGAP1/Rho/ERK

signaling axis, leading to early relapse in HCC. Especially, in patients with HBV-associated HCC, high RACGAP1 expression was linked with an increased risk of mortality [36].

ASPM is the human ortholog of the Drosophila melanogaster abnormal spindle gene, which is required for

proper mitotic spindle activity in embryonic neuroblasts. It has been shown to play a role in tumor development. The overexpression of the gene was proven to be a genetic marker indicating HCC's increased invasive/metastatic potential, a greater likelihood of early tumor recurrence independent of p53 mutation status or tumor stage, and hence a poor prognosis [37]. The N6-methyladenosine modification of ASPM mRNA mediated by METTL3 promoted its expression in liver HCC [38]. ASPM also promotes tumor growth by antagonizing autophagy-mediated Dvl2 degradation and boosting Wnt/-catenin signaling [39].

This study had several following limitations. First, we only used a gene expression profile from the GEO database; this dataset just covers mRNA expression and does not include microRNA, lncRNA, or circRNA expression. This maybe narrows the identification of other potential biomarkers in HBV HCC. Furthermore, additional experiments, both in vivo and in vitro, are required to demonstrate the predictive usefulness of these hub genes.

In conclusion, in the present research, 119 DEGs were found in HBV-associated HCC. Five hub genes, CCNB1, CDK1, TOP2A, RACGAP1, and ASPM, have been identified as potential prognostic biomarkers and therapeutic targets for HBV-associated HCC.

Acknowledgements

References

1. Villanueva A. Hepatocellular Carcinoma. The New England Journal of Medicine. 2019 04 11;380(15):1450-1462. https://doi.org/10.1056/NEJMra1713263

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018 Nov;68(6):394-424. https://doi.org/10.3322/caac.21492

3. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nature Reviews. Gastroenterology & Hepatology. 2019 Oct;16(10):589-604. https://doi.org/10.1038/s41575-019-0186-y

4. Tang X, Allain J, Wang H, Rong X, Chen J, Huang K, Xu R, Wang M, Huang J, Liao Q, Shan Z, Luo S, Li T, Li C, Fu Y. Incidence of hepatitis B virus infection in young Chinese blood donors born after mandatory implementation of neonatal hepatitis B vaccination nationwide. Journal of Viral Hepatitis. 2018 09;25(9):1008-1016. https://doi.org/10.1111/jvh.12901

5. Whitford K, Liu B, Micallef J, Yin JK, Macartney K, Van Damme P, Kaldor JM. Long-term impact of infant immunization on hepatitis B prevalence: a systematic review and meta-analysis. Bulletin of the World Health Organization. 2018 07 01;96(7):484-497. https://doi.org/10.2471/BLT.17.205153

6. Xie D, Ren Z, Zhou J, Fan J, Gao Q. Critical appraisal of Chinese 2017 guideline on the management of hepatocellular carcinoma. Hepatobiliary Surgery and Nutrition. 2017 Dec;6(6):387-396. https://doi.org/10.21037/hbsn.2017.11.01

7. Marrero J, Kulik L, Sirlin C, Zhu A, Finn R, Abecassis M, Roberts L, Heimbach J. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. Hepatology (Baltimore, Md.). 2018 08;68(2). https://doi.org/10.1002/hep.29913

8. Liu M, Liu M, Tang T. Association between chemotherapy and prognostic factors of survival in hepatocellular carcinoma: a SEER population-based cohort study. Scientific reports. 2021 Dec 09;11(1). https://doi.org/10.1038/s41598-021-02698-x

9. Tang J, Luo Y, Wu G. A glycolysis-related gene expression signature in predicting recurrence of breast cancer. Aging (Albany NY). 2020 Nov 16;12(24):24983-24994. https://doi.org/10.18632/aging.103806

10. Hoang TM, Bui TTH, Nguyen TT. Comprehensive Analysis of the Expression and Prognosis for DCTPP1 gene in Breast Cancer. Asian Pacific Journal of Cancer Biology. 2021 07 09;6(3):195-200. https://doi.org/10.31557/apjcb.2021.6.3.195-200

11. Yin L, Jie G, Fengkai X, Qiaoliang Z, Di G, Chunlai L. Transcriptomic and functional network features of lung squamous cell carcinoma through integrative analysis of GEO and TCGA data. Scientific Reports. 2018 Oct 26;8(1):15834. https://doi.org/10.1038/s41598-018-34160-w

12. Sun Q, Li X, Xu M, Zhang L, Zuo H, Xin Y, Zhang L, Gong P. Differential Expression and Bioinformatics Analysis of circRNA in Non-small Cell Lung Cancer. Frontiers in Genetics. 2020 Nov 24;11:586814. https://doi.org/10.3389/fgene.2020.586814

13. Chen L, Lu D, Sun K, Sun Y, Hu P, Li X, Xu F. Identification of biomarkers associated with diagnosis and prognosis of colorectal cancer patients based on integrated bioinformatics analysis. Gene. 2019 04 15;692. https://doi.org/10.1016/j.gene.2019.01.001

14. Ren N, Liang B, Li Y. Identification of prognosis-related genes in the tumor microenvironment of stomach adenocarcinoma by TCGA and GEO datasets. Bioscience Reports. 2020 Oct 13;40(10):BSR20200980. https://doi.org/10.1042/BSR20200980

15. Wu J, Huang Y, Yu C, Li X, Wang L, Hong J, Lin D, Han X, Guo G, Hu T, Huang H. The Key Gene Expression Patterns and Prognostic Factors in Malignant Transformation from Enchondroma to Chondrosarcoma. Frontiers in Oncology. 2021 09 10;11:693034. https://doi.org/10.3389/fonc.2021.693034

16. Hoang TM, Nguyen MT, Chen WS, Zhuang CY, Wang ZX, Wang HQ, et al. Elevated expression of SKP2 correlates with poor prognosis in osteosarcoma: a bioinformatics analysis. Biomed Res Ther, 2021; 8(12), 4782-92.

17. Wang S, Ooi L, Hui K. Identification and Validation of a Novel Gene Signature Associated with the Recurrence of Human Hepatocellular Carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2007 Dec 01;13:6275-83. https://doi.org/10.1158/1078-0432.CCR-06-2236

18. Snel B, Lehmann G, Bork P, Huynen M. STRING: a web-server to retrieve and display the repeatedly occurring neighbourhood of a gene. Nucleic acids research. 2000 09 15;28(18). https://doi.org/10.1093/nar/28.18.3442

19. Chia-Hao C, Shu-Hwa C, Hsin-Hung W, Chin-Wen H, Ming-Tat K, Chung-Yen L. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Systems Biology. 2014 Dec 08;8(4):S11. https://doi.

org/10.1186/1752-0509-8-S4-S11

20. Tang Z, Chenwei L, Kang B, Gao G, Cheng L, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Research. 2017 07 03;45(W1):W98-W102. https://doi.org/10.1093/nar/gkx247

21. Yang X, Miao B, Wei C, Dong R, Gao P, Zhang X, Lu J, Gao C, Wang X, Sun H, Zhou J, Fan J, Ai-Wu K, Guo-Ming S, Jia-Bin C. Lymphoid-specific helicase promotes the growth and invasion of hepatocellular carcinoma by transcriptional regulation of centromere protein F expression. Cancer Science. 2019 07;110(7):2133-2144. https://doi.org/10.1111/cas.14037

22. Suzuki T, Urano T, Miki Y, Moriya T, Akahira J, Ishida T, Horie K, Inoue S, Sasano H. Nuclear cyclin B1 in human breast carcinoma as a potent prognostic factor. Cancer Science. 2007 05;98(5):644-651. https://doi.org/10.1111/j.1349-7006.2007.00444.x

23. Ding L, Yang L, Yuqi H, Bingtao Z, Fangli R, Xuanzi F, Wang Y, Mengdi L, Jun L, Kuang Y, Sihan L, Zhai W, Danhui M, Yanfang J, Quentin L, Baoqing J, Sheng J, Chang Z. CREPT/RPRD1B associates with Aurora B to regulate Cyclin B1 expression for accelerating the G2/M transition in gastric cancer. Cell Death & Disease. 2018 Dec 05;9(12):1172. https://doi.org/10.1038/s41419-018-1211-8

24. Hou Y, Wang X, Wang J, Sun X, Liu X, Hu H, Fan W, Zhang X, Wu D. Cyclin B1 acts as a tumor microenvironment-related cancer promoter and prognostic biomarker in hepatocellular carcinoma. The Journal of International Medical Research. 2021 05;49(5):3000605211016265. https://doi.org/10.1177/03000605211016265

25. Junsheng G, Xiaorui L, Juan L, Yuting H. MicroRNA-144 inhibits cell proliferation, migration and invasion in human hepatocellular carcinoma by targeting CCNB1. Cancer Cell International. 2019;19:15. https://doi.org/10.1186/s12935-019-0729-x

26. Peng X, Luo R, Li J, He A, Wang X, Wan H, Cai Y, Dong W, Lin J. Zingiberene targets the miR-16/cyclin-B1 axis to regulate the growth, migration and invasion of human liver cancer cells. Journal of B.U.ON.: official journal of the Balkan Union of Oncology. 2020 08;25(4):1904-1910. https://doi.org/10.1186/s12935-019-0729-x

27. Weng L, Du J, Zhou Q, Cheng B, Li J, Zhang D, Ling C. Identification of cyclin B1 and Sec62 as biomarkers for recurrence in patients with HBV-related hepatocellular carcinoma after surgical resection. Molecular Cancer. 2012 06 08;11:39. https://doi.org/10.1186/1476-4598-11-39

28. Hao L, Li S, Peng Q, Guo Y, Ji J, Zhang Z, Xue Y, Liu Y, Shi X. Anti-malarial drug dihydroartemisinin downregulates the expression levels of CDK1 and CCNB1 in liver cancer. Oncology Letters. 2021 09;22(3):653. https://doi.org/10.3892/ol.2021.12914

29. Wu CX, Wang XQ, Chok SH, Man K, Tsang SHY, Chan ACY, Ma KW, Xia W, Cheung TT. Blocking CDK1/PDK1/β-Catenin signaling by CDK1 inhibitor RO3306 increased the efficacy of sorafenib treatment by targeting cancer stem cells in a preclinical model of hepatocellular carcinoma. Theranostics. 2018;8(14):3737-3750. https://doi.org/10.7150/thno.25487

30. Beiwei Y, Chen L, Zhang W, Yue L, Zhang Y, Gao Y, Teng X, Wang Q, Jia H, Liu X, Zheng H, Hou P, Yu H, Sun Y, Zhang Z, Zhang P, Zhang L. TOP2A and CENPF are synergistic master regulators activated in cervical cancer. BMC Medical Genomics. 2020 Oct 06;13(1):145. https://doi.org/10.1186/s12920-020-00800-2

31. Liu T, Zhang H, Yi S, Lubing G, Zhou M. Mutual regulation of MDM4 and TOP2A in cancer cell proliferation. Molecular Oncology. 2019 05;13(5):1047-1058. https://doi.org/10.1002/1878-0261.12457

32. Labbé D, Sweeney C, Brown M, Galbo P, Rosario S, Wadosky K, Sjöström M, et al. TOP2A and EZH2 Provide Early Detection of an Aggressive Prostate Cancer Subgroup. Clinical cancer research : an official journal of the American Association for Cancer Research. 2017 Nov 15;23(22). https://doi.org/10.1158/1078-0432.CCR-17-0413

33. Panvichian R, Tantiwetrueangdet A, Angkathunyakul N, Leelaudomlipi S. TOP2A amplification and overexpression in hepatocellular carcinoma tissues. BioMed Research International. 2015;2015:381602. https://doi.org/10.1155/2015/381602

34. Liao S, Wang K, Zhang L, Shi G, Wang Z, Chen Z, Zhu P, He Q. PRC1 and RACGAP1 are Diagnostic Biomarkers of Early HCC and PRC1 Drives Self-Renewal of Liver Cancer Stem Cells. Frontiers in Cell and Developmental Biology. 2022;10.

35. Wang M, Chen D, Qi B, Li M, Zhu Y, Yin W, He L, Yu Y, Li Z, Lin L, Yang F, Lin Z, Liu J. Pseudogene RACGAP1P activates RACGAP1/Rho/ERK signalling axis as a competing endogenous RNA to promote hepatocellular carcinoma early recurrence. Cell Death & Disease. 2019 06 03;10(6):426. https://doi.org/10.1038/s41419-019-1666-2

36. Sha M, Cao J, Zong Z, Xu N, Zhang J, Tong Y, Xia Q. Identification of genes predicting unfavorable prognosis in hepatitis B virus-associated hepatocellular carcinoma. Annals of Translational Medicine. 2021 06;9(12):975. https://doi.org/10.21037/atm-21-2085

37. Lin S, Pan H, Liu S, Jeng Y, Hu F, Peng S, Lai P, Hsu H. ASPM is a novel marker for vascular invasion, early recurrence, and poor prognosis of hepatocellular carcinoma. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research. 2008 08 01;14(15):4814-4820. https://doi.org/10.1158/1078-0432.CCR-07-5262

38. Wang A, Chen X, Li D, Yang L, Jiang J. METTL3-mediated m6A methylation of ASPM drives hepatocellular carcinoma cells growth and metastasis. Journal of Clinical Laboratory Analysis. 2021 09;35(9). https://doi.org/10.1002/jcla.23931

39. Zhang H, Yang X, Zhu L, Li Z, Zuo P, Wang P, et al. ASPM promotes hepatocellular carcinoma progression by activating Wnt/β-catenin signaling through antagonizing autophagy-mediated Dvl2 degradation. FEBS open bio. 2021 Oct;11(10). https://doi.org/10.1002/2211-5463.13278