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Identifying a Three-Gene Signature and Associated Drugs for Hepatitis B Virus-Related Hepatocellular Carcinoma Using Comprehensive Bioinformatics Analysis

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Liver cancer is one of the most common cancer forms and a significant contributor to global cancer-associated mortality. Hepatitis B virus (HBV) infection contributes enormously to HCC development and progression. Despite this, the molecular basis of liver tumorigenesis is not clear. This work focused on identifying the relevant genetic markers and available drugs for treating HBV-related HCC. Differentially expressed genes (DEGs) from HBV-related HCC samples and corresponding healthy samples were identified from GSE62232 and GSE121248 datasets from the GEO2R repository (Gene Expression Omnibus). The Venn diagram software screened the overlapping DEGs between these two datasets. The DEGs were functionally assessed using protein-protein interaction (PPI), Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses by different bioinformatic methods, and hub genes were screened. Hub genes and related drugs were verified by the GEPIA2 (Gene Expression Profiling Interactive Analysis) web server and the Quartata Web online platform. Overall, 116 DEGs (88 up-regulated and 28 down-regulated) related to signal transduction and metabolic pathways were identified. The nine significant target hub genes were *TOP2A, RRM2, DTL, ECT2, ASPM, ANLN, BUB1B, CCNB1,* and *CDK1*. Moreover, one screened drug, Fostamatinib, was targeted to *CDK1*. Our study identified three genes and associated drugs as probable targets for studying HBV-related HCC.

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Introduction

Hepatocellular carcinoma (HCC) represents recurrent primary liver cancer (LC) with a poor prognosis (Kim et al. 2017). Several factors like hepatitis, diabetes, smoking, and alcohol consumption lead to HCC (Fujiwara et al. 2018). Hepatitis B virus (HBV) infection is one of the most important causes of HCC and has been involved in 66% of cases (Stanaway et al. 2016). Despite technological improvements in diagnosing and treating HCC, HCC cases with HBV infection show dismal prognosis because of genetic alterations resulting from virus infection, irreversible liver injury, and cirrhosis (Burton et al. 2022). The last decade has seen significant progress in diagnosing, preventing, and developing treatment modalities such as surgical resection, chemotherapy, radiotherapy, and individualized, targeted therapies. However, HCC has a low 5-year overall survival (OS), primarily due to its invasive nature. Also, its histological features are not characterized molecularly, and approaches to targeted therapy are lacking (Tang et al. 2020). Therefore, early identification of genomic alterations and therapeutic drugs for HBV-related HCC (HBV-HCC) is essential.

The use of high-throughput sequencing has increased dramatically recently, especially in cancer biology (Hosseini et al. 2022). Data from clinical studies and sequencing experiments are available in many public databases. Bioinformatic methods provide new insights into tumors by reanalyzing these data. Most bioinformatic analyses on HBV-HCC focus on mining a single gene signature for these tumors (Wu et al. 2019; Sha et al. 2021). Nevertheless, one individual gene is inadequate for under-

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standing the tumor since carcinogenesis involves multiple factors, stages, and genes. Furthermore, such research neglects the genes as well as targeted drugs. Consequently, HBV-HCC continues to be challenging to diagnose and has a poor prognosis for patients.

Drug repurposing or repositioning is a revolutionary breakthrough in developing new drugs because it discovers novel applications of used therapeutics (Roy et al. 2021). New anti-tumor drug discovery and development are time-consuming and costly. According to systems biology, drugs developed for non-cancer indications can target crucial molecules in cancer progression (Kale et al. 2021). Researchers interested in establishing a tumor therapy explore the option of repurposing old molecules that have gained approval from the Food and Drug Administration (FDA) and those applied safely in the clinic. For example, thalidomide was initially developed to treat pregnancy-related nausea in the 1950s. Later, it was suggested as the monotherapy for multiple myeloma because of its antiangiogenic property. Finally, thalidomide was approved by the FDA in 2006 for patients with multiple refractory myelomas, making it a classic repurposed drug. Therefore, a hypothesis was put forth to check if anti-tumor drugs for HBV-HCC could be identified by bioinformatic screening of existing drugs.

The present study used integrated bioinformatics to identify the possible markers and drugs to treat HBV-HCC. First, two microarray datasets from the Gene Expression Omnibus (GEO) database were selected and analyzed. After that, differentially expressed genes (DEGs) were identified in tumor and healthy samples. Next, protein-protein interaction (PPI) network, Gene Ontology (GO) functional annotation, and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed among the DEGs using bioinformatics tools. As a result, potential biomarkers, pathway markers, and drugs associated with HBV-HCC were screened. The potential genes and the related drugs discovered in this work offer possible features to diagnose and treat HBV-HCC.

Materials and Methods

Microarray data

The NCBI-GEO databases identified DEGs in HBV-HCC samples compared with healthy hepatic samples. GSE62232 and GSE121248 were acquired from the GEO database by adopting the GPL570 platform (GeneChip Human Genome U133 Plus 2.0 Array, Affymetrix, Santa Clara, CA, USA). Specifically, the GSE62232 dataset contained ten HBV-HCC samples and ten healthy hepatic samples. The GSE121248 dataset included 70 chronic hepatitis-mediated HCC samples and 37 corresponding healthy hepatic samples.

Screening for differentially expressed genes (DEGs)

Statistical cut-off criteria of $P < 0.05$ and $|\log FC| \geq 2$ were used for screening the DEGs. After that, Venn Software was adopted for screening the intersected DEGs. The $\log FC \leq -2$ represents the down-regulated genes among these DEGs, and the $\log FC \geq 2$ represents the up-regulated genes.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses

GO analysis identifies biological characteristics based on high-throughput transcriptomic and genomic data (Sha et al. 2021). Genomes, biological pathways, enzymes, drugs, compounds, and diseases were interpreted in KEGG, an integrated database resource. The GO enrichment and KEGG pathways were analyzed using DAVID (Database for Annotation, Visualization, and Integrated Discovery), a web-based bioinformatics tool. Statistical significance was determined by a $P < 0.05$ cut-off.

Establishment of the protein-protein interaction (PPI) network and selection of hub genes

An open website called STRING database [Search Tool for Retrieval of Interacting Genes (https://string-db.org)] was adopted for assessing and integrating the interactions of DEGs (Szklarczyk et al. 2021). The PPI network was visualized and analyzed using the Cytoscape software Version 3.9.0 (https://www.cytoscape.org/). Besides, the ten most significantly up-regulated and down-regulated hub genes were selected by the cytoHubba plugin (scores > 2) (Chin et al. 2014).

Prognosis analysis of the hub genes in GEPIA2

The website GEPIA2 (http://gepia2.cancer-pku.cn) provides an interactive approach for analyzing the predictive value of the hub genes. Besides, Kaplan-Meier (KM) curves were utilized to determine the overall survival (OS) for the hub genes.

Building drug-gene interactions

The Quartata Web (http://quartata.csb.pitt.edu/) allows users to explore drug-gene interactions. This server identified the hub genes uploaded into a database and screened them against existing drugs and compounds.

Statistical analysis

The moderated t-test was used to identify the DEGs. $P < 0.05$ indicated statistical significance.

Results

Identification of the DEGs

GEO2R identified the DEGs from two databases, GSE62232 (524) and GSE12248 (114). The intersection of these two databases resulted in 116 genes (including 28 up-regulated and 88 down-regulated) presented in the Venn Diagram (Fig. 1, Table 1).

Analysis of GO and KEGG

In the Biological Process (BP) category in GO, DEGs

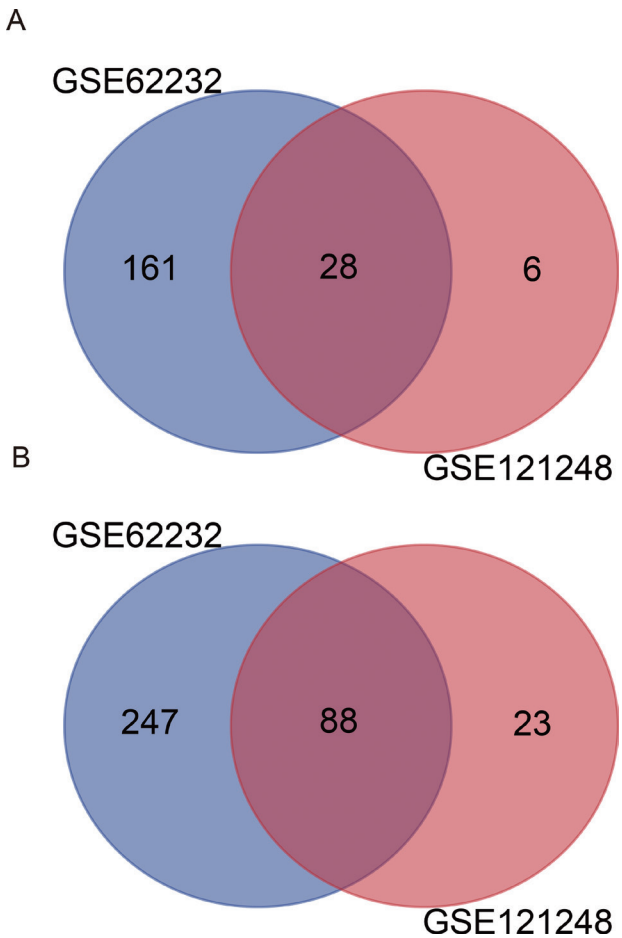

Fig. 1. A total of 116 differentially expressed genes (DEGs) were found in the two databases (GSE62232 and GSE12248).
(A) Common 28 up-regulated DEGs. (B) Common 88 down-regulated DEGs.

were principally enriched in "signal transduction," "xenobiotic metabolic process," and "cell adhesion." In the Cellular Component (CC) category in GO, DEGs mainly were associated with "extracellular region," "extracellular exosome," and "extracellular space." In the Molecular Function (MF) category, "heme binding," "monooxygenase activity," and "iron ion binding" genes were clustered. Moreover, the genes in the KEGG pathway were primarily associated with "Retinol metabolism," "metabolism of cytochrome P450," and "Metabolic pathways" (Table 2).

Construction and analysis of PPIs and hub genes

The network shows up-regulated 78 genes/nodes and 446 edges (Fig. 2A). The ten most significant ones included *TOP2A, RRM2, DTL, ECT2, ASPM, ANLN, BUB1B, CDKN3, CCNB1,* and *CDK1*, which were all up-regulated genes (Fig. 2B). Default settings were established for all parameters in cytoHubba.

Survival analysis and identification of the hub genes

The expression analysis of the top ten genes was conducted by uploading them on the GEPIA2 website and verifying their relation with tumors. These ten genes showed significantly higher expression in liver tumors compared to normal liver tissues (Fig. 3A). Moreover, survival analysis indicated a significant association between the overall survival (OS) rate and the following nine genes: *TOP2A, RRM2, DTL, ECT2, ASPM, ANLN, BUB1B, CCNB1,* and *CDK1*. The *CDKN3* gene showed no statistical significance. The high expression level of these nine hub genes was related to an increased mortality risk among HBV-HCC cases (Fig. 3B).

The construction of the drug-gene interaction

The nine hub genes exhibiting significant association with overall survival were imported to Quartata Web to analyze the interactions between the drugs and the genes. The three genes, *TOP2A, RRM2,* and *CDK1*, matched with predicted drugs, while the remaining six hub genes did not have any matching targets (Table 3). (1) *TOP2A* matched 38 known interaction drugs (the FDA approves 24 drugs of this group) and 20 predicted drugs. The following 11 drugs were identified: Mitoxantrone, Etoposide, Amsacrine,

Table 1. The identified differentially expressed genes (DEGs).

DEGs	Gene name
Up-regulated	*CDK1, SPINK1, CAP2, DTL, RACGAP1, CTHRC1, IGF2BP3, RRM2, CCNB1, TOP2A, ASPM, HMMR, CDKN3, AKR1B10, PBK, GPC3, SULT1C2, ROBO1, SPP1, ZIC2, NEK2, ANLN, ACSL4, CRNDE, BUB1B, COL15A1, ECT2, PRC1*
Down-regulated	*CYP4A22, BBOX1, CYP26A1, CYP2A6, CNTN3, TENM1, LINC01093, CXCL14, SLC22A1, IGF1, SULT1E1, CYP39A1, HAO2, FAM134B, MT1F, SLC25A47, MFSD2A, ZG16, FLJ22763, HHIP, KCNN2, ZGPAT, SLCO1B3, CYP1A2, CNDP1, BCO_2, ACSM3, FCN3, GBA3, TTC36, CLEC4G, C3P1, CDH19, CYP2B6, GYS2, FOLH1B, KMO, CD5L, LPA, GHR, CLEC1B, CXCL2, MIR675, FOSB, LIFR, FAM65C, CYP2C9, CLRN3, CYP2A7, LCAT, CLEC4M, VNN1, ESR1, PLAC8, HAMP, ALDOB, DNASE1L3, DCN, NAT2, BCHE, IL1RAP, AKR1D1, CXCL12, TMEM27, CRHBP, THRSP, IDO2, HGFAC, IGFALS, ADGRG7, C7, ZGPAT, FREM2, ADH4, GPM6A, OIT3, MT1M, HGF, GLYAT, CYP2B7P, GLS2, SRD5A2, ADRA1A, APOF, C9, SRPX, FCN2, LINC00844*

Table 2. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of the DEGs.

Category	Term	Counts	Ratio	P-value
BP[1]	GO:0007165~signal transduction	14	12.612613	0.0133363
	GO:0006805~xenobiotic metabolic process	8	7.2072072	1.56E-06
	GO:0007155~cell adhesion	8	7.2072072	0.0304675
CC[2]	GO:0005576~extracellular region	31	27.927928	1.73E-07
	GO:0005615~extracellular space	26	23.423423	1.01E-05
	GO:0070062~extracellular exosome	19	17.117117	0.0341874
MF[3]	GO:0020037~heme binding	9	8.1081081	1.82E-06
	GO:0005506~iron ion binding	9	8.1081081	1.03E-06
	GO:0004497~monooxygenase activity	7	6.3063063	2.33E-06
KEGG	hsa00830: Retinol metabolism	7	6.3063063	1.18E-05
	hsa01100: Metabolic pathways	26	23.423423	7.11E-05
	hsa00982:Drug metabolism-cytochrome P450	6	5.4054054	2.09E-04

BP[1], Biological Process; CC[2], Cellular Component; MF[3], Molecular Function.

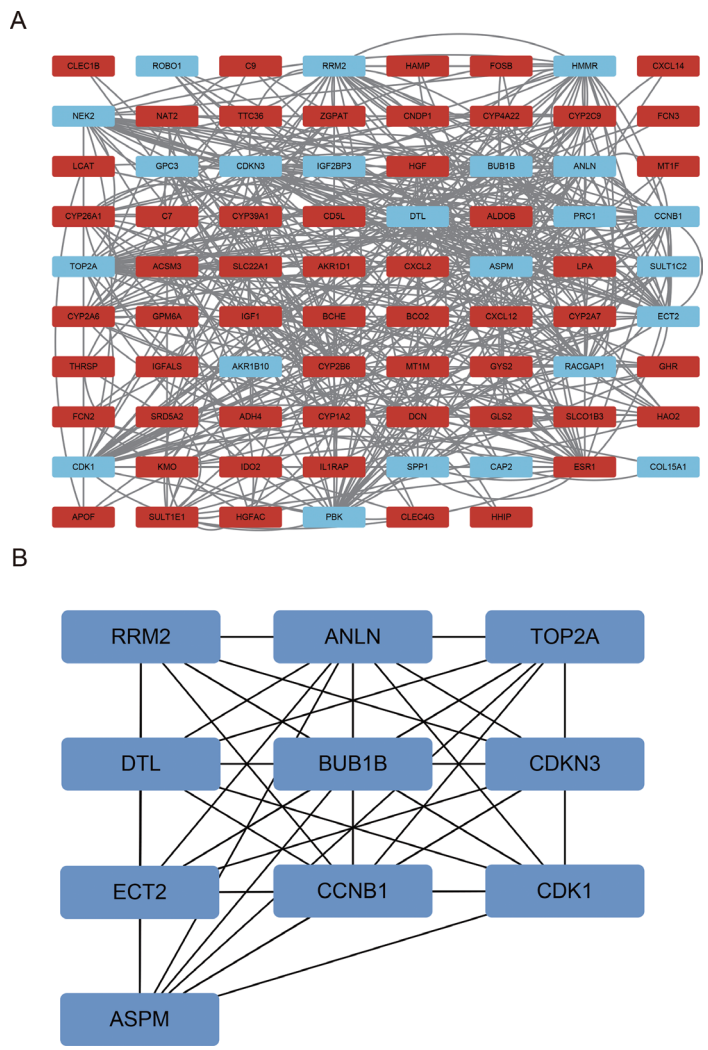

Fig. 2. Construction of protein-protein interaction (PPI) network and hub genes analysis.
(A) PPI networks of differentially expressed genes. (B) Top10 genes in the PPI works. The genes in blue boxes represent the up-regulated genes while those in red boxes represent down-regulated genes.

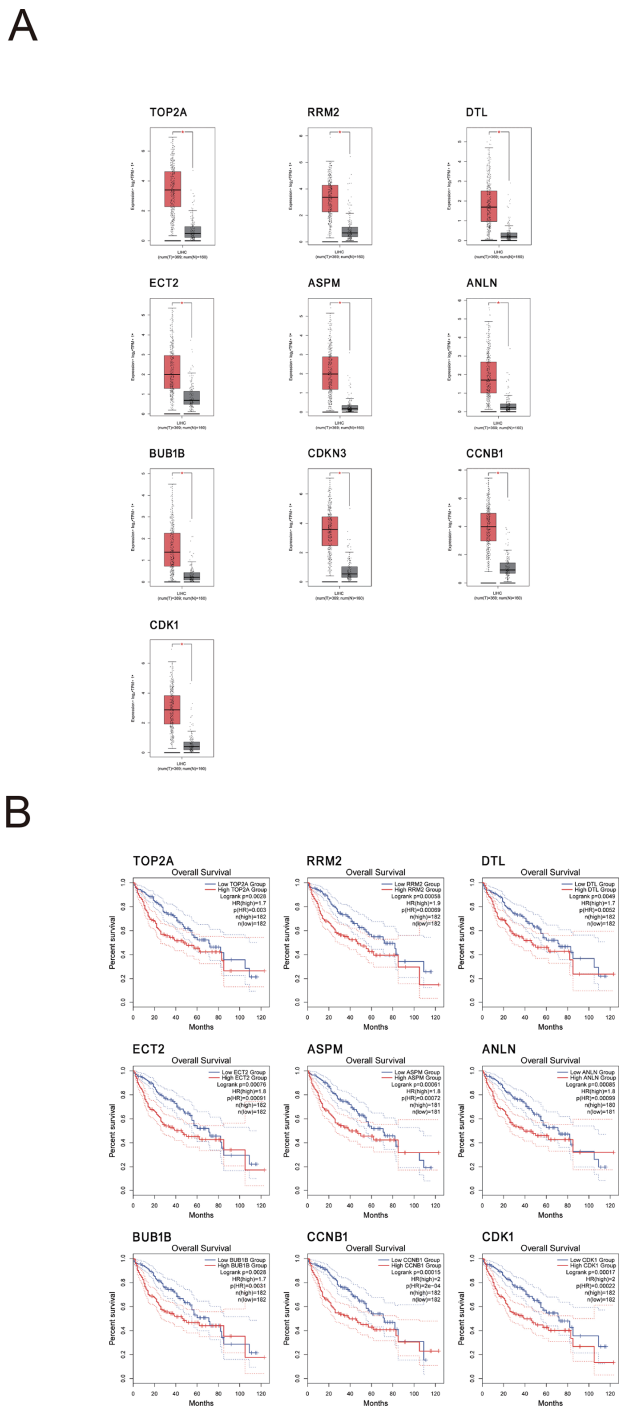

Fig. 3. The expression levels of top10 genes in liver tumor and normal tissue samples and the statistical overall survival (OS) rate of hub genes.
(A) Boxplot showing the expression levels of top10 genes in tumor and normal tissues of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) patients. *P < 0.05. (B) OS rate of hub genes in HBV-related HCC patients.

Epirubicin, Valrubicin, Idarubicin, Doxorubicin, Amrubicin, Daunorubicin, Teniposide, Dactinomycin. (2) *RRM2* was identified and matched with five known interaction drugs and 20 predicted drugs. Two known interaction drugs, Cladribine and Gallium nitrate, are approved by the FDA. (3) *CDK1* was identified and matched with nine known interaction drugs and 20 predicted drugs; only Fostamatinib is approved by FDA (Table 3).

Discussion

Human cancer, including liver cancer, is among the top five killers and a significant public health concern. Approximately 240 million patients suffer from HBV infection globally. This infection is the second most common cause of cancer after tobacco and a major contributor to liver cancer. HCC treatment has changed over the past two decades. There is a safe vaccine against HBV, yet HBV is a major contributor to global chronic liver disease. Consequently, identifying creditable biomarkers to diagnose and treat HBV-HCC is crucial. Bioinformatics has developed rapidly, generating vast sequencing data and multiple microarrays, which can be used to unravel mechanisms for diagnosis and prognosis, and associated drugs of varied diseases, especially cancers (Wooller et al. 2017; Li et al. 2020).

The present study examined GSE62232 and GSE12248 datasets using the GEO2R database repository to identify the DEGs in HBV-HCC samples compared with healthy controls. Altogether 116 DEGs, including 28 up-regulated and 88 down-regulated, were selected. The DEGs in the Biological Process (BP) category were mostly associated with protein metabolic processes, such as "xenobiotic metabolic process" and "signal transduction." The DEGs in the Cellular Component (CC) category were mostly associated with "extracellular area," like "extracellular exosome," "extracellular space," "extracellular region," and they were tightly associated with the extracellular microenvironment. The DEGs in the Molecular Function (MF) class were enriched into "heme binding," "monooxygenase activity," "iron ion binding" terms, and they were tightly associated with the cell-extracellular matrix interaction. The genes up-regulated in the KEGG pathways were mostly associated with metabolic issues, such as "retinol metabolism" and "metabolic pathways." They were closely related to tumor formation, and "drug metabolism of cytochrome P450."

The constructed PPI network exhibited 78 genes/nodes and 446 edges associated with the DEGs. Next, the ten most significant hub genes were selected using the cytoHubba software. Patients with HBV-HCC displayed a higher expression of *TOP2A, RRM2, DTL, ECT2, ASPM, ANLN, BUB1B, CCNB1,* and *CDK1* genes. The overall survival rate of such patients was lower, and they significantly showed a poor prognosis.

Topoisomerase II alpha (*TOP2A*), a DNA topoisomerase, is involved in replication and transcription by altering the topological structures of DNA. *TOP2A* shows aberrant expression in diverse cancer types, such as prostate, gastric, colonic, breast, ovarian, and head and neck cancers. Moreover, *TOP2A* was identified as one of the hub genes through network, survival, and PPI network analyses

Table 3. The significant drugs targeted to hub genes.

Gene	Drug ID	Drug name	Drug type	Drug group
TOP2A	DB01204	Mitoxantrone	Small Molecule Drug	Approved; Investigational
	DB00773	Etoposide	Small Molecule Drug	Approved
	DB00276	Amsacrine	Small Molecule Drug	Approved; Investigational
	DB00445	Epirubicin	Small Molecule Drug	Approved
	DB00385	Valrubicin	Small Molecule Drug	Approved
	DB01177	Idarubicin	Small Molecule Drug	Approved
	DB00997	Doxorubicin	Small Molecule Drug	Approved; Investigational
	DB06263	Amrubicin	Small Molecule Drug	Approved; Investigational
	DB00694	Daunorubicin	Small Molecule Drug	Approved
	DB00444	Teniposide	Small Molecule Drug	Approved
	DB00970	Dactinomycin	Small Molecule Drug	Approved; Investigational
RRM2	DB00242	Cladribine	Small Molecule Drug	Approved; Investigational
	DB05260	Gallium nitrate	Small Molecule Drug	Approved; Investigational
CDK1	DB12010	Fostamatinib	Small Molecule Drug	Approved; Investigational

(Nguyen et al. 2021). TOP2A directly interacts with p53, the extensively recognized tumor suppressor protein. Thus, a comprehensive investigation identified TOP2A as a candidate marker for diagnosing and predicting the prognosis of HBV-HCC (Liao et al. 2019).

RRM2 is the Ribonucleotide reductase subunit M2. Its over-expression enhances E-cadherin and Bcl-2 pathway activation and decreases p53 pathway activation (Jin et al. 2020). RRM2 up-regulation independently predicts dismal prognostic outcomes for lung adenocarcinoma cases. In liver cancer, RRM2 resists ferroptosis by maintaining GSH production. Serum RRM2 functions as a biomarker for evaluating the ferroptosis suppression level and improving the diagnostic efficiency of liver cancer (Yang et al. 2020). Some RRM2-targeting drugs like Pterostilbene simultaneously inhibit HBV replication and HCC proliferation; this mode of action can be a novel application to treat HBV-HCC and HCC (Wang et al. 2021).

DTL (denticleless E3 ubiquitin-protein ligase homolog) is a critical tumorigenic and prognostic regulator. *DTL* gene has been associated with several cancers and tumor-promoting activity. DTL regulates the AKT/mTOR axis and can be used to predict the prognosis and accelerate the progression of bladder cancer (Luo et al. 2022). DTL is the candidate therapeutic target for anti-tumor metastasis in cervical adenocarcinoma (Liu et al. 2021). Targeting DTL inhibits TPX2. This induces senescence and arrest of the HCC cell cycle while suppressing clone formation and cell proliferation (Chen et al. 2018).

Epithelial Cell Transforming Sequence 2 (ECT2) is an oncogene involved in human cancers. *ECT2* was initially recognized as a proto-oncogene while selecting murine keratinocyte cDNA expression library of genes that transformed mouse fibroblasts. Additionally, ECT2 is overexpressed in numerous human cancers (Chen et al. 2015; Wang et al. 2016).

Abnormal spindle microtubule assembly (ASPM) represents the centrosomal protein, which plays a crucial role in mitotic spindle regulation, neurogenesis, and brain size regulation. Over-expression of ASPM in various cancers is associated with a poor clinical outcome and recurrence. ASPM inhibits cell cycle arrest during G2/M, predicting increased invasiveness and metastasis in HCC (Lin et al. 2008). Different bioinformatic methods identified a reduction in G2/M cell cycle arrest when ASPM was over-expressed. Thus, it was a biomarker for HCC with enhanced invasive/metastatic potential. These literature findings are similar to those obtained in the present study.

ANLN (anillin) represents the actin-binding protein with an essential role in cleavage furrow assembly in the process of cytokinesis (Lian et al. 2018). ANLN enhances cancer proliferation by reducing DNA injury and apoptosis. Inhibiting ANLN within liver cells suppresses cytokinesis and inhibits liver cancer progression (Zhang et al. 2018).

BUB1B (BubR1) is encoded by the *BUB1B* gene. It is an integral part of the spindle assembly checkpoint during mitosis and postpones the anaphase onset while guaranteeing appropriate chromosome segregation (Zhang et al. 2021). BUB1B enhances HCC development by activating the mTORC1 pathway (Fu et al. 2016). In patients with HCC, BUB1B overexpression is closely related to unfavorable clinical characteristics, poor OS, and reduced recurrence-free survival (RFS) (Qiu et al. 2020).

Similarly, bioinformatics methods identified that CDK1 and CCNB1 could predict disease prognosis and are related to immunocyte infiltration within HCC, which correlates with various immune markers (Zou et al. 2020). CDK1 (cyclin-dependent kinase 1) interacts with HBV_circ_1 for regulating cell growth within HBV-HCC (Zhu et al. 2021).

The present work selected hub genes associated with previously identified "old" drugs to discover new anti-tumor therapeutics using bioinformatics. The "old" drugs are those approved by FDA or with safe clinical applica-

tions. This study demonstrated that *TOP2A, RRM2,* and *CDK1* matched with predicted drugs approved by FDA.

TOP2A matched with 24 drugs, and 11 of them are used for cancer therapy. Among these drugs, Mitoxantrone is the chemotherapeutic agent used for treating worsening relapsing-remitting, progressive, or secondary progressive multiple sclerosis (Kamm et al. 2014). Etoposide is a podophyllotoxin derivative used for treating small cell lung cancer (SCLC) and testicular cancer (Zhao et al. 2021). Amsacrine is cytotoxic and is utilized for inducing remission among acute myeloid leukemia (AML) cases with no adequate treatment response (Krejci et al. 2013). Epirubicin is an anthracycline topoisomerase II inhibitor used to treat axillary node metastases in cases with surgically resected primary breast cancer (Moammeri et al. 2022). Valrubicin, one of the anthracyclines, is applied via the bladder to treat bladder carcinoma with BCG resistance (Cookson et al. 2014). Idarubicin is an anthracycline anti-neoplastic agent used for adult AML. Doxorubicin is a drug adopted for treating Kaposi's Sarcoma and other cancers (Liu et al. 2018). Amrubicin is a synthetic anthracycline currently being developed to treat small-cell lung cancer (Sato et al. 2021). Daunorubicin, an anthracycline aminoglycoside, induces remission in acute lymphocytic leukemia (ALL) and non-lymphocytic leukemia (Tsimberidou et al. 2003). Teniposide is a cytotoxic drug used to induce chemotherapy in refractory ALL in children. Dactinomycin, one of the actinomycins, treats different cancer types (Langholz et al. 2011; Hadi et al. 2020).

RRM2 matched with two drugs named Cladribine and Gallium nitrate. The purine antimetabolite Cladribine is used to manage relapsing multiple sclerosis when alternative multiple sclerosis drugs are ineffective or the patient cannot tolerate them (Signori et al. 2020). Gallium nitrate is indicated for hypercalcemia caused by cancer (Leyland-Jones 2004).

CDK1 matched with only one drug named Fostamatinib, the oral small-molecule inhibitor for a spleen tyrosine kinase (SYK) used to treat chronic immune thrombocytopenia. Abnormal activation of SYK is tightly associated with the occurrence of hematological malignancy. Thus, drug targeting has emerged as the research hotspot. Currently, this mechanism of fostamatinib is under investigation for treating several autoimmune diseases (Tang et al. 2022). It effectively inhibited prostate cancer cell proliferation (Wang et al. 2020). Thus, it can be used as a target to resist cancer cells with high invasive capacity (Bacolod and Barany 2021), such as colon cancer (Hu et al. 2020). Moreover, Fostamatinib was identified as a valuable supplement to sorafenib. It can be used as a possible first-line therapy or after sorafenib resistance, alone or in combination (Regan-Fendt et al. 2020).

The active metabolite of fostamatinib, R406, suppresses signaling via the Fcγ receptors (Braselmann et al. 2006), and it also destroys platelets during chronic immune thrombocytopenic purpura (ITP) through antibodies (Riccaboni et al. 2010). R406 suppresses the activation of B and T lymphocytes via B-cell receptors and T-cell receptors, respectively (Sedlik et al. 2003). Moreover, the knock-on effect of inhibiting signal transduction is observed from the production of cytokines and inflammatory factors, such as leukotriene C4, tumor necrosis factor, granular cytokines, and interleukin-8 (Sanderson et al. 2010). Thus, R406 shows anti-cancer characteristics and can be the new anti-HCC therapeutic target.

In summary, the following nine genes, *TOP2A, RRM2, DTL, ECT2, ASPM, ANLN, BUB1B, CCNB1,* and *CDK1,* were identified as possible diagnostic and prognostic biomarkers. The three genes, *TOP2A, RRM2, CDK1,* and their potential associated drugs, were chosen to explore new avenues in treating HBV-HCC. The substantial bias risk limited the current study, and the drugs need to be verified in the future by relevant experimental models.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

References

Bacolod, M.D. & Barany, F. (2021) A unified transcriptional, pharmacogenomic, and gene dependency approach to decipher the biology, diagnostic markers, and therapeutic targets associated with prostate cancer metastasis. *Cancers* (*Basel*), **13**, 5158.

Braselmann, S., Taylor, V., Zhao, H., Wang, S., Sylvain, C., Baluom, M., Qu, K., Herlaar, E., Lau, A., Young, C., Wong, B.R., Lovell, S., Sun, T., Park, G., Argade, A., et al. (2006) R406, an orally available spleen tyrosine kinase inhibitor blocks fc receptor signaling and reduces immune complex-mediated inflammation. *J. Pharmacol. Exp. Ther.*, **319**, 998-1008.

Burton, A., Balachandrakumar, V.K., Driver, R.J., Tataru, D., Paley, L., Marshall, A., Alexander, G., Rowe, I.A.; HCC-UK/BASL/NCRAS Partnership, Palmer, D.H. & Cross, T.J.S. (2022) Regional variations in hepatocellular carcinoma incidence, routes to diagnosis, treatment and survival in England. *Br. J. Cancer*, **126**, 804-814.

Chen, J., Xia, H., Zhang, X., Karthik, S., Pratap, S.V., Ooi, L.L., Hong, W. & Hui, K.M. (2015) ECT2 regulates the Rho/ERK signalling axis to promote early recurrence in human hepatocellular carcinoma. *J. Hepatol.*, **62**, 1287-1295.

Chen, Y.C., Chen, I.S., Huang, G.J., Kang, C.H., Wang, K.C., Tsao, M.J. & Pan, H.W. (2018) Targeting DTL induces cell cycle arrest and senescence and suppresses cell growth and colony formation through TPX2 inhibition in human hepatocellular carcinoma cells. *Onco Targets Ther.*, **11**, 1601-1616.

Chin, C.H., Chen, S.H., Wu, H.H., Ho, C.W., Ko, M.T. & Lin, C.Y. (2014) cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst. Biol.*, **8** Suppl 4, S11.

Cookson, M.S., Chang, S.S., Lihou, C., Li, T., Harper, S.Q., Lang, Z. & Tutrone, R.F. (2014) Use of intravesical valrubicin in clinical practice for treatment of nonmuscle-invasive bladder cancer, including carcinoma in situ of the bladder. *Ther. Adv. Urol.*, **6**, 181-191.

Fu, X., Chen, G., Cai, Z.D., Wang, C., Liu, Z.Z., Lin, Z.Y., Wu,

Y.D., Liang, Y.X., Han, Z.D., Liu, J.C. & Zhong, W.D. (2016) Overexpression of BUB1B contributes to progression of prostate cancer and predicts poor outcome in patients with prostate cancer. *Onco Targets Ther.*, **9**, 2211-2220.

Fujiwara, N., Friedman, S.L., Goossens, N. & Hoshida, Y. (2018) Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. *J. Hepatol.*, **68**, 526-549.

Hadi, L.M., Yaghini, E., MacRobert, A.J. & Loizidou, M. (2020) Synergy between photodynamic therapy and dactinomycin chemotherapy in 2D and 3D ovarian cancer cell cultures. *Int. J. Mol. Sci.*, **21**, 3203.

Hosseini, K., Ranjbar, M., Pirpour Tazehkand, A., Asgharian, P., Montazersaheb, S., Tarhriz, V. & Ghasemnejad, T. (2022) Evaluation of exosomal non-coding RNAs in cancer using high-throughput sequencing. *J. Transl. Med.*, **20**, 30.

Hu, D., Zhang, B., Yu, M., Shi, W. & Zhang, L. (2020) Identification of prognostic biomarkers and drug target prediction for colon cancer according to a competitive endogenous RNA network. *Mol. Med. Rep.*, **22**, 620-632.

Jin, C.Y., Du, L., Nuerlan, A.H., Wang, X.L., Yang, Y.W. & Guo, R. (2020) High expression of RRM2 as an independent predictive factor of poor prognosis in patients with lung adenocarcinoma. *Aging* (*Albany N. Y.*), **13**, 3518-3535.

Kale, V.P., Habib, H., Chitren, R., Patel, M., Pramanik, K.C., Jonnalagadda, S.C., Challagundla, K. & Pandey, M.K. (2021) Old drugs, new uses: drug repurposing in hematological malignancies. *Semin. Cancer Biol.*, **68**, 242-248.

Kamm, C.P., Uitdehaag, B.M. & Polman, C.H. (2014) Multiple sclerosis: current knowledge and future outlook. *Eur. Neurol.*, **72**, 132-141.

Kim, D.W., Talati, C. & Kim, R. (2017) Hepatocellular carcinoma (HCC): beyond sorafenib-chemotherapy. *J. Gastrointest. Oncol.*, **8**, 256-265.

Krejci, M., Doubek, M., Dusek, J., Brychtova, Y., Racil, Z., Navratil, M., Tomiska, M., Horky, O., Pospisilova, S. & Mayer, J. (2013) Combination of fludarabine, amsacrine, and cytarabine followed by reduced-intensity conditioning and allogeneic hematopoietic stem cell transplantation in patients with high-risk acute myeloid leukemia. *Ann. Hematol.*, **92**, 1397-1403.

Langholz, B., Skolnik, J.M., Barrett, J.S., Renbarger, J., Seibel, N.L., Zajicek, A. & Arndt, C.A. (2011) Dactinomycin and vincristine toxicity in the treatment of childhood cancer: a retrospective study from the Children's Oncology Group. *Pediatr. Blood Cancer*, **57**, 252-257.

Leyland-Jones, B. (2004) Treating cancer-related hypercalcemia with gallium nitrate. *J. Support. Oncol.*, **2**, 509-516.

Li, H., Pei, F., Taylor, D.L. & Bahar, I. (2020) QuartataWeb: integrated chemical-protein-pathway mapping for polypharmacology and chemogenomics. *Bioinformatics*, **36**, 3935-3937.

Lian, Y.F., Huang, Y.L., Wang, J.L., Deng, M.H., Xia, T.L., Zeng, M.S., Chen, M.S., Wang, H.B. & Huang, Y.H. (2018) Anillin is required for tumor growth and regulated by miR-15a/miR-16-1 in HBV-related hepatocellular carcinoma. *Aging* (*Albany N. Y.*), **10**, 1884-1901.

Liao, X., Yu, T., Yang, C., Huang, K., Wang, X., Han, C., Huang, R., Liu, X., Yu, L., Zhu, G., Su, H., Qin, W., Deng, J., Zeng, X., Han, B., et al. (2019) Comprehensive investigation of key biomarkers and pathways in hepatitis B virus-related hepatocellular carcinoma. *J. Cancer*, **10**, 5689-5704.

Lin, S.Y., Pan, H.W., Liu, S.H., Jeng, Y.M., Hu, F.C., Peng, S.Y., Lai, P.L. & Hsu, H.C. (2008) ASPM is a novel marker for vascular invasion, early recurrence, and poor prognosis of hepatocellular carcinoma. *Clin. Cancer Res.*, **14**, 4814-4820.

Liu, R., Zhou, J., Yang, S. & Zhang, Z. (2018) Efficacy and safety of pegylated liposomal doxorubicin-based chemotherapy of AIDS-related Kaposi's sarcoma: a meta-analysis. *Am. J. Ther.*, **25**, e719-e721.

Liu, S., Gu, L., Wu, N., Song, J., Yan, J., Yang, S., Feng, Y., Wang, Z., Wang, L., Zhang, Y. & Jin, Y. (2021) Overexpression of DTL enhances cell motility and promotes tumor metastasis in cervical adenocarcinoma by inducing RAC1-JNK-FOXO1 axis. *Cell Death Dis.*, **12**, 929.

Luo, Y., He, Z., Liu, W., Zhou, F., Liu, T. & Wang, G. (2022) DTL is a prognostic biomarker and promotes bladder cancer progression through regulating the AKT/mTOR axis. *Oxid. Med. Cell. Longev.*, **2022**, 3369858.

Moammeri, A., Abbaspour, K., Zafarian, A., Jamshidifar, E., Motasadizadeh, H., Dabbagh Moghaddam, F., Salehi, Z., Makvandi, P. & Dinarvand, R. (2022) pH-responsive, adorned nanoniosomes for codelivery of cisplatin and epirubicin: synergistic treatment of breast cancer. *ACS Appl. Bio. Mater.*, **5**, 675-690.

Nguyen, T.B., Do, D.N., Nguyen-Thanh, T., Tatipamula, V.B. & Nguyen, H.T. (2021) Identification of five hub genes as key prognostic biomarkers in liver cancer via integrated bioinformatics analysis. *Biology* (*Basel*), **10**, 957.

Qiu, J., Zhang, S., Wang, P., Wang, H., Sha, B., Peng, H., Ju, Z., Rao, J. & Lu, L. (2020) BUB1B promotes hepatocellular carcinoma progression via activation of the mTORC1 signaling pathway. *Cancer Med.*, **9**, 8159-8172.

Regan-Fendt, K., Li, D., Reyes, R., Yu, L., Wani, N.A., Hu, P., Jacob, S.T., Ghoshal, K., Payne, P.R.O. & Motiwala, T. (2020) Transcriptomics-based drug repurposing approach identifies novel drugs against sorafenib-resistant hepatocellular carcinoma. *Cancers* (*Basel*), **12**, 2730.

Riccaboni, M., Bianchi, I. & Petrillo, P. (2010) Spleen tyrosine kinases: biology, therapeutic targets and drugs. *Drug Discov. Today*, **15**, 517-530.

Roy, S., Dhaneshwar, S. & Bhasin, B. (2021) Drug repurposing: an emerging tool for drug reuse, recycling and discovery. *Curr. Drug Res. Rev.*, **13**, 101-119.

Sanderson, M.P., Gelling, S.J., Rippmann, J.F. & Schnapp, A. (2010) Comparison of the anti-allergic activity of Syk inhibitors with optimized Syk siRNAs in FcepsilonRI-activated RBL-2H3 basophilic cells. *Cell. Immunol.*, **262**, 28-34.

Sato, Y., Iihara, H., Kinomura, M., Hirose, C., Fujii, H., Endo, J., Yanase, K., Kaito, D., Sasaki, Y., Gomyo, T., Sakai, C., Iwai, M., Tsuboi, Y., Ishihara, T., Kobayashi, R., et al. (2021) Primary prophylaxis of febrile neutropenia with pegfilgrastim in small-cell lung cancer patients receiving amrubicin as second-line therapy. *Anticancer Res.*, **41**, 1615-1620.

Sedlik, C., Orbach, D., Veron, P., Schweighoffer, E., Colucci, F., Gamberale, R., Ioan-Facsinay, A., Verbeek, S., Ricciardi-Castagnoli, P., Bonnerot, C., Tybulewicz, V.L., Di Santo, J. & Amigorena, S. (2003) A critical role for Syk protein tyrosine kinase in Fc receptor-mediated antigen presentation and induction of dendritic cell maturation. *J. Immunol.*, **170**, 846-852.

Sha, M., Cao, J., Zong, Z.P., Xu, N., Zhang, J.J., Tong, Y. & Xia, Q. (2021) Identification of genes predicting unfavorable prognosis in hepatitis B virus-associated hepatocellular carcinoma. *Ann. Transl. Med.*, **9**, 975.

Signori, A., Sacca, F., Lanzillo, R., Maniscalco, G.T., Signoriello, E., Repice, A.M., Annovazzi, P., Baroncini, D., Clerico, M., Binello, E., Cerqua, R., Mataluni, G., Perini, P., Bonavita, S., Lavorgna, L., et al. (2020) Cladribine vs other drugs in MS: merging randomized trial with real-life data. *Neurol. Neuroimmunol. Neuroinflamm.*, **7**, e878.

Stanaway, J.D., Flaxman, A.D., Naghavi, M., Fitzmaurice, C., Vos, T., Abubakar, I., Abu-Raddad, L.J., Assadi, R., Bhala, N., Cowie, B., Forouzanfour, M.H., Groeger, J., Hanafiah, K.M., Jacobsen, K.H., James, S.L., et al. (2016) The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. *Lancet*, **388**, 1081-1088.

Szklarczyk, D., Gable, A.L., Nastou, K.C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N.T., Legeay, M., Fang, T., Bork, P., Jensen, L.J. & von Mering, C. (2021) The STRING database in 2021: customizable protein-protein networks, and func-

tional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.*, **49**, D605-D612.

Tang, S., Yu, Q. & Ding, C. (2022) Investigational spleen tyrosine kinase (SYK) inhibitors for the treatment of autoimmune diseases. *Expert Opin. Investig. Drugs*, **31**, 291-303.

Tang, Y., Zhang, Y. & Hu, X. (2020) Identification of potential hub genes related to diagnosis and prognosis of hepatitis B virus-related hepatocellular carcinoma via integrated bioinformatics analysis. *Biomed. Res. Int.*, **2020**, 4251761.

Tsimberidou, A.M., Kantarjian, H.M., Cortes, J., Thomas, D.A., Faderl, S., Garcia-Manero, G., Verstovsek, S., Ferrajoli, A., Wierda, W., Alvarado, Y., O'Brien, S.M., Albitar, M., Keating, M.J. & Giles, F.J. (2003) Fractionated cyclophosphamide, vincristine, liposomal daunorubicin, and dexamethasone plus rituximab and granulocyte-macrophage-colony stimulating factor (GM-CSF) alternating with methotrexate and cytarabine plus rituximab and GM-CSF in patients with Richter syndrome or fludarabine-refractory chronic lymphocytic leukemia. *Cancer*, **97**, 1711-1720.

Wang, H.B., Yan, H.C. & Liu, Y. (2016) Clinical significance of ECT2 expression in tissue and serum of gastric cancer patients. *Clin. Transl. Oncol.*, **18**, 735-742.

Wang, J., Wang, L., Chen, S., Peng, H., Xiao, L., Du, E., Liu, Y., Lin, D., Wang, Y., Xu, Y. & Yang, K. (2020) PKMYT1 is associated with prostate cancer malignancy and may serve as a therapeutic target. *Gene*, **744**, 144608.

Wang, R., Xu, Z., Tian, J., Liu, Q., Dong, J., Guo, L., Hai, B., Liu, X., Yao, H., Chen, Z., Xu, J., Zhu, L., Chen, H., Hou, T., Zhu, W., et al. (2021) Pterostilbene inhibits hepatocellular carcinoma proliferation and HBV replication by targeting ribonucleotide reductase M2 protein. *Am. J. Cancer Res.*, **11**, 2975-2989.

Wooller, S.K., Benstead-Hume, G., Chen, X., Ali, Y. & Pearl, F. M.G. (2017) Bioinformatics in translational drug discovery. *Biosci. Rep.*, **37**, BSR 20160180.

Wu, M., Liu, Z., Zhang, A. & Li, N. (2019) Identification of key genes and pathways in hepatocellular carcinoma: a preliminary bioinformatics analysis. *Medicine* (*Baltimore*), **98**, e14287.

Yang, Y., Lin, J., Guo, S., Xue, X., Wang, Y., Qiu, S., Cui, J., Ma, L., Zhang, X. & Wang, J. (2020) RRM2 protects against ferroptosis and is a tumor biomarker for liver cancer. *Cancer Cell Int.*, **20**, 587.

Zhang, P., Feng, J., Wu, X., Chu, W., Zhang, Y. & Li, P. (2021) Bioinformatics analysis of candidate genes and pathways related to hepatocellular carcinoma in China: a study based on public databases. *Pathol. Oncol. Res.*, **27**, 588532.

Zhang, S., Nguyen, L.H., Zhou, K., Tu, H.C., Sehgal, A., Nassour, I., Li, L., Gopal, P., Goodman, J., Singal, A.G., Yopp, A., Zhang, Y., Siegwart, D.J. & Zhu, H. (2018) Knockdown of anillin actin binding protein blocks cytokinesis in hepatocytes and reduces liver tumor development in mice without affecting regeneration. *Gastroenterology*, **154**, 1421-1434.

Zhao, W., Cong, Y., Li, H.M., Li, S., Shen, Y., Qi, Q., Zhang, Y., Li, Y.Z. & Tang, Y.J. (2021) Challenges and potential for improving the druggability of podophyllotoxin-derived drugs in cancer chemotherapy. *Nat. Prod. Rep.*, **38**, 470-488.

Zhu, M., Liang, Z., Pan, J., Zhang, X., Xue, R., Cao, G., Hu, X. & Gong, C. (2021) Hepatocellular carcinoma progression mediated by hepatitis B virus-encoded circRNA HBV_circ_1 through interaction with CDK1. *Mol. Ther. Nucleic Acids*, **25**, 668-682.

Zou, Y., Ruan, S., Jin, L., Chen, Z., Han, H., Zhang, Y., Jian, Z., Lin, Y., Shi, N. & Jin, H. (2020) CDK1, CCNB1, and CCNB2 are prognostic biomarkers and correlated with immune infiltration in hepatocellular carcinoma. *Med. Sci. Monit.*, **26**, e925289.