Genes Associated with Prognosis of Hepatocellular Carcinoma

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Research

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Abstract

Background: Hepatocellular carcinoma (HCC) is the foremost common malignant liver malady within the world. In any case, the unthinking relationship between each quality and the signaling pathway remains vague. In this think about, we pointed to explain the potential center candidate genes and pathways for HCC.

Methods: The GSE41804, GSE62232 and GSE65372 expression profiles cover 139 tumors and 46 non tumors and can be downloaded from the Gene Expression Integrated Database (GEO). Furthermore, we also downloaded the RNA arrangement information set of liver cancer from the cancer genome map (TCGA) database. Then use the online database David 6.8 The Kyoto Encyclopedia of Genes and Genomic Pathways. The protein-protein interaction organized complexes of these Differentially expressed genes (DEGs) were developed by utilizing cytochrome program, atomic complex discovery plug-in and online database STRING.

Results: To begin with, we identified 84 abnormally expressed genes (39 upregulation genes and 45 downregulation genes) that were abnormally expressed in both the Earth synthetic orbit and the TCGA dataset. In expansion, 10 center qualities were recognized: CCNB1, CCNA2, NCAPG, AURKA, ASPM, TOP2A, DLGAP5, NUSAP1, NUF2 and RACGAP1. At long last, the by and large survival examination and relationship examination were performed for these qualities.

Conclusions: These discoveries recommend that center qualities and pathways recognized through bioinformatics examination can incredibly enhance our understanding of HCC improvement and repeat. In expansion, these candidate qualities and pathways may be restorative targets for HCC treatment.

Background

Hepatocellular carcinoma (HCC) is one of the cancers with the highest incidence in the world and the third leading cause of cancer death in the world ¹. HCC represents a complex multistep disease involving processes involving genome amplification, deletion, insertion, or mutation to induce a range of epigenetic and genetic changes. With the development of science and technology, the operation and treatment have been updated, especially in the early diagnosis and interventional treatment of liver cancer. However, for most patients with advanced HCC, the 5-year survival rate is low and the prognosis is poor²⁻⁴. Although people have constantly updated the treatment methods, techniques and concepts of HCC, and considered various potential treatment methods and techniques, they are not significantly effective. The atomic pathogenesis of this illness is amazingly complex and different, counting highlights such as quality variety, quality expression, and genome-wide methylation⁵. Hence, it is fundamental to get it the exact atomic components of HCC event, dissemination, and repeat, so as to create successful symptomatic and helpful methodologies ⁶.

With the rapid development and application of high-throughput technology in recent years, the biomedical research on liver cancer is increasing. Gene chip can be utilized to quickly distinguish the differentially communicated qualities of tumor within the expression data of the same test at time focuses (DEGs)⁷. Although the research is progressing continuously, there are false positive rates in the free microarray survey, which makes it difficult to obtain reliable results. Therefore, people can get more reliable results by applying Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Protein-Protein Interaction (PPI). Through a series of screening, we obtained 84 differentially expressed genes, and screened out 10 pivotal genes from these 84 differentially expressed genes, which may affect the diagnosis and treatment of liver cancer and have important value. We aim to uncover new insights into the biological characteristics and pathogenesis of HCC and to provide a conceptual perspective for the development and progress of HCC.

Methods

Microarray data

Three quality expression profiles were gotten from the NCBI geosynchronous orbit database: GSE41804, GSE62232 and GSE65372. The GSE41804 dataset is based on GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array, Affymetrix, Santa Clara, Ca). The GSE62232 dataset is based on the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array). The GSE65372 dataset is based on the GPL14951 platform (Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip). These three data sets contained 185 samples, including 139 tumor samples and 46 non-tumor samples. All methods in this ponder accommodate to the convention.

Data processing

Geo2R could be a free download framework for online information examination in GEO; We selected three gene sets, namely GSE41804, GSE62232 and GSE65372, from which all the liver cancer tissues and normal liver tissues were included. We built up the taking after incorporation criteria for DEGs: up-regulated qualities must have a $\log_2$ -fold alter ($\log FC$) ≥ 2 and an balanced P value < 0.05 , whereas down-regulated qualities must have a $\log FC \leq -2$ and an balanced P value < 0.05 . In this way, Venn charts of up-regulated and down-regulated qualities were plotted for distinctive databases.

GO enrichment and pathway analysis

Go database, the full name of which is Gene Ontology, divides the functions of genes into three parts: Cellular component (CC), molecular function (MF), biological process (BP). Using the GO database, we can get what our target genes are mainly associated with at the three levels of CC, MF and BP. Through Kyoto Encyclopedia of Genes and Genomics (KEGG), we can search various known metabolic pathways and associate related genes. We used DAVID, a networkable program that combines functional genome annotations with intuitive graphical summaries to see the GO and KEGG enrichment of DEGs. A P value < 0.05 was considered statistically significant.

PPI networks and module analysis

The search device utilized to recover the association genes (STRING, adaptation 11.0) database was utilized to recover DEG-encoded protein and PPI organize data. The database contains 24.6 million proteins and 2 billion intuitive including 5,090 living beings. we transfer DEG to the STRING database, and 0.900 (the highest confidence in the cross score) is set as a valid threshold. Then, Construction of PPI network with Cytoscape software⁸. Built in atomic complex location (mcode) in Cytoscape is an robotized strategy for analyzing exceedingly interconnected modules such as atomic complexes or clusters. Examination parameters are set by default. Two critical quality modules were distinguished by utilitarian improvement examination of DEGs, with $P < 0.05$ as the edge.

Verification of hub genes

To confirm mRNA expression levels of central qualities screened from HCC and non-tumor liver tissues, the UALCAN microarray database (<http://ualcan.path.uab.edu/index.html>) was utilized ⁹. UALCAN could be a comprehensive, user-friendly, and intelligently web asset for analyzing cancer OMICS information. It is built on PERL-CGI with tall quality illustrations utilizing javascript and CSS. UALCAN is planned to, a) give simple get to to freely accessible cancer OMICS information (TCGA and MET500), b) permit clients to distinguish biomarkers or to perform in silico approval of potential qualities of intrigued, c) give charts and plots delineating quality expression and quiet survival data based on quality expression, d) assess quality expression in atomic subtypes of breast and prostate cancer, e) assess epigenetic direction of quality expression by promoter methylation and relate with quality expression, f) perform pan-cancer quality expression examination, and g) Give extra data around the chosen genes/targets by connecting to HPRD, GeneCards, Pubmed, TargetScan, The human protein chart book, DRUGBANK, Open Targets and the GTEx. These assets permit analysts to accumulate important data and information around the genes/targets of intrigued.

Survival analysis of core genes

Prognostic data for the 10 center qualities is accessible within the free online UALCAN database (accessible online: <http://ualcan.path.uab.edu/index.html>). As appeared in Fig. 5, irregular expression levels of ten center qualities are related with destitute guess of hepatocellular carcinoma.

Results

DEG recognition

Through GSE41804, GSE62232 and GSE65372. Venn analysis is performed to check the intersection between the DEG contours. Among them, 84 DEGs were determined from three profile data sets(Figure 1A). It should be noted that compared with non-cancerous liver tissues, 45 DEGs were significantly down-regulated in HCC tissues, while 39 DEGs were significantly up-regulated.

PPIN construction and hub gene identification

Using STRING database to determine 84 PPI pairs in DEG. As shown in Fig. 1B. The top ten central genes were identified according to how connected they were. Results show that the cyclin B1 (CCNB1) is the most critical genes, the maximum core is 11.715, followed by cyclin A2 (CCNA2), its core is 11.681, lectin compound subunits (NCAPG) degree of 11.635, Aurora kinase (AURKA) core is 11.622, abnormal spindle microtubule assembly(ASPM) core is 11.613,DNA topoisomerase II alpha (TOP2A) core is 11.593, DLG associated protein 5 (DLGAP5) core is 11.579,nucleoli and spindle related protein 1 (NUSAP1) core in 11.524, NUF2 component of NDC80 kinetochore complex (NUF2) core is 11.427, Rac GTPase activating protein 1 (RACGAP1) core is 11.424 (Table 1). Ppins for ten distinguished center qualities were moreover developed, showing solid intelligent between each other (Fig. 1c).

Table 1

Top 10 in network ppi.txt ranked by EPC method		
Rank	Name	Score
1	CCNB1	11.715
2	CCNA2	11.681
3	NCAPG	11.635
4	AURKA	11.622
5	ASPM	11.613
6	TOP2A	11.593
7	DLGAP5	11.579
8	NUSAP1	11.524
9	NUF2	11.427
10	RACGAP1	11.424

GO annotation and enrichment analysis of KEGG pathway

In order to further understand the biological function of these 84 DEG, enricher database was used(<http://amp.pharm.mssm.edu/Enrichr/>). We further analyzed the enrichment of differential genes by GO and KEGG pathways. Figure 2A shows the results of GO and KEGG enrichment analysis of 10 hub genes selected by us. The results showed that 10 genes were mainly enriched in progesterone-mediated oocyte mating and cellular senility (Fig. 2A). The first four significantly rich MF terms include Pathways in cancer, Cell cycle, Metabolic pathways, Human T-cell leukemia virus 1 infection, Progesterone-mediated oocyte maturation, Cellular senescence, FoxO signaling pathway, Viral carcinogenesis and Hepatocellular carcinoma (Fig. 2b).

Ten pivot genes were screened out

The expression level of a pivotal gene (CCNB1, CCNA2, NCAPG, AURKA, ASPM, TOP2A, DLGAP5, NUSAP1, NUF2, RACGAP1) in liver cancer tissues and non-tumor liver tissues was analyzed. UALCAN database. As appeared in Fig. 3, mRNA expression level of (Fig. 3A) CCNB1, (Fig. 3B) CCNA2, (Fig. 3C) CCNB2, (Fig. 3D) NCAPG, (Fig. 3E) PBK (Fig. 3B). Figure 3F) Compared with non-cancer tissues, NUCC1 (Fig. 3G) AURKA, (Fig. 3H) ZWINT, (Fig. 3I) PRC1 and (Fig. 3J) KIF4A were essentially up-regulated in HCC tissues ($P < 0.05$).

Survival analysis of hepatocellular carcinoma central genes

The 10 PPI chosen central qualities were assist analyzed by OS and DFS utilizing UALCAN database, bioinformatics examination and GEPIA database. As appeared in Fig. 4, tall expression levels of CCNB1, CCNA2, NCAPG, AURKA, ASPM, TOP2A, DLGAP5, NUSAP1, NUF2 and RACGAP1 in liver cancer patients were related with destitute OS. Only the P value of NUSAP1 was greater than 0.05.

Discussion

HCC related qualities have not been recognized to illustrate the potential atomic components of cancer defenselessness, movement and forecast. That's to say, unused treatments focusing on coordinate administrative instruments are still required, which are related with destitute forecast in HCC patients. In this consider, we chosen three common microarray datasets from Asian nations, specifically GSE41804, GSE62232 and GSE65372. Concurring to the incorporation criteria between 139 HCC tests and 46 non-cancerous tests, 86 DEG were screened with geo2r. The PPI organize of DEGs is utilized to identify central genes, and after that the part of these central qualities in HCC is confirmed by OS examination of patients in ualcan. The expression of these central qualities was compared between HCC and non-cancerous tissues of TCGA and GTEX. At last, 10 Central qualities related with destitute forecast of HCC were distinguished: CCNB1, CCNA2, NCAPG, AURKA, ASPM, TOP2A, DLGAP5, NUSAP1, NUF2 and RACGAP1.

CCNB1 as a helpful approach for HCC¹⁰, is straightforwardly repressed by Mir-144. Subsequently, CCNB1 expression is frequently utilized to assess the guess after treatment with anticancer drugs. Cyclin B1 (CCNB1) shapes a complex with CDK1 (cyclin subordinate kinase 1) to control the G2 / M stage of the mammalian cell cycle, which plays an vital part within the start of mitosis¹¹.

NUF2 is a centromere-related protein¹². It plays the most important role in cell proliferation and apoptosis, affecting the combination of centromere and spindle microtubules, and also participating in cell cycle regulation¹³. NUF2 is overexpressed in various cancers (lung cancer, cholangiocarcinoma, renal cell carcinoma and bladder cancer)¹⁴. Although some studies have suggested that NUF2 is highly expressed in breast cancer and has an important prognosis^{14,15}, Its exact function and potential molecular mechanism remain to be studied.

Human panda associated protein 5 (DLGAP5) gene, found on chromosome 14q22.3, may be a cell cycle controller included in carcinogenesis¹⁶. Wang et al. detailed that DLGAP5 was up-regulated in non-small cell lung cancer (NSCLC) and was related with a shorter survival time. In cytological tests, knockdown DLGAP5 restrained NSCLC cell multiplication, movement, and intrusion¹⁷. Past thinks about have too appeared that up-regulated DLGAP5 expression is related with destitute forecast in bladder cancer¹⁸, prostate cancer¹⁹, liver cancer²⁰, and leukemia²¹.

NCAPG is the administrative subunit of the coagulation complex, which is related to the cell cycle, mitosis and cell cycle chromosome concentration, which changes over interchange chromatin to mitotic chromosome concentration amid mitosis and meiosis²². Although NCAPG may be a new carcinogen, its role in liver cancer has not been widely studied. NCAPG hindrance can restrain the development and expansion of HCC cells^{23,24} and repress the development of HuH7 and HCCLM3 tumor xenografts²³. It is found that NCAPG is over-expressed in HCC, which is helpful to cancer recurrence and decrease survival rate of HCC patients^{23,24}. In liver cancer patients, the overexpression of CCNB1 was positively correlated with the overexpression of NCAPG²³. Through bioinformatics analysis, NCAPG has been identified as the pivotal gene of HCC²⁵⁻²⁷. The comes about of this consider propose that expanded expression of NCAPG may lead to destitute forecast for HCC patients. Hence, NCAPG plays a critical part in HCC movement and serves as a unused restorative target for moving forward HCC treatment.

AURKA could be a mitotic serine/threonine kinase related with the control of mitosis, cell division, and cell cycle movement²⁸. The clinical part of AURKA in liver cancer has been broadly considered. AURKA overexpression was recognized in HCC cell lines²⁹ and HCC tissue samples^{30,31}. AURKA overexpression is strongly associated with aggressive tumor characteristics of HCC³², poor prognosis³¹, and chemical resistance³³. The disclosure of AURKA quality polymorphisms recommends that AURKA may serve as a prescient biomarker for early HCC³⁴. Another robotic consider appeared that AURKA can advance HCC metastasis by directing epithelial-mesenchymal change and carcinoma stem-like characteristics³¹. In HCC cells, transcriptional AURKA was uncovered to be directed by C-MYC, which advances the HCC handle³⁵. Restraint of AURKA by Alisertib³⁶, a compound right now being tried in stage II/III clinical trials in patients with hematologic malignancies and strong tumors, may diminish reasonability and initiate apoptosis of HCC cells²⁹. Based on TCGA survival and expression information, Zhou et al. revealed a negative correlation between AURKA overexpression and OS²⁷. The show ponder too appeared that the expanded AURKA expression level was related with destitute OS and DFS in HCC patients.

Related studies have shown that CCNA2 is also overexpressed in human hepatocellular carcinoma. In addition, CCNA2 can reduce the overall survival rate and increase the risk of tumor recurrence in patients with HCC³⁷. What can be determined is that HCC patients with high expression of CCNA2 have longer OS and DFS than HCC patients with high expression of CCNA2.

Related studies have shown that TOP2A is also overexpressed in HCC tissues³⁸, It is highly consistent with our results. In addition, some studies have shown that the overexpression of TOP2A affects the

occurrence of malignant tumors and chemotherapy resistance, thus shortening the survival time of patients³⁹. The changes of DNA structure will occur in the transcription process of TOP2A, which further affects the early onset of HCC, microvascular invasion, shortened survival time of patients, and the relationship between chemotherapy resistance and recurrence. Relevant studies have proved that^{40,41}. Related studies have shown that TOP2A is the target of anti-tumor drugs such as epirubicin, adriamycin, etoposide and temozolomide^{42–44}. TOP2A is often overexpressed simultaneously with HER2 in breast cancer⁴⁵. However, there is no correlation between the overexpression of TOP2A and HER2, and the overexpression of Top2A in HCC does not depend on the amplification or overexpression of HER2⁴⁶. More studies have found that it is overexpressed in lung cancer, colon cancer and ovarian cancer, so there is reason to believe its value and has far-reaching significance for cancer prediction and treatment^{47–49}.

It is found that the high expression of NUSAP1 is related to the progression of prostate cancer, which may be related to its regulation of mitosis⁵⁰. In addition, it was found that NUSAP1 may be a microtubule-associated protein, which further controls cell cycle by promoting microtubule aggregation⁵¹. It was profoundly upregulated in different tumor sorts, counting prostate cancer⁵², pancreatic cancer⁵³, and invasive breast cancer⁵⁴. In liver cancer patients, NUSAP1 expression was found to be up-regulated, and its overexpression may be a prognostic factor⁵⁵. In the mouse model of liver cancer, NUSAP1 knockdown reduced the proliferation, migration and survival of liver cancer cells⁵⁵. In this think about, the current discoveries moreover uncover that expanded NUSAP1 expression is related with destitute OS and DFS in HCC patients.

Conclusion

In summary, 10 HCC-related central genes were identified using three cohort data sets and integrated bioinformatics analysis. Expression of central genes was found to increase in liver cancer, and overexpression levels predicted poor prognosis. Advance ponders with a bigger test measure ought to be conducted to confirm the current discoveries. In expansion, it is fundamental to supply exploratory prove to explore the useful part of distinguished qualities in liver cancer. It is trusted that this consider will contribute to the disclosure of novel demonstrative and prognostic biomarkers and restorative targets for liver cancer.

Abbreviations

HCC

Hepatocellular carcinoma; GEO:Gene Expression Integrated Database; TCGA:The Cancer Genome Atlas; KEGG:Kyoto Encyclopedia of Genes and Genomic Pathways; GO:Gene Ontology; DEGs:Differentially expressed genes; BP:Biological Processes; MF:Molecular Functions; CC:Cellular Components; OS:Overall Survival.

Declarations

Ethics approval and consent to participate

Ethical Approval is not applicable for this article.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WG wrote the manuscript. ZC provided expert advice. All authors read and approved the final version.

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References

1. Cui T, Liu Y, Wang J, Liu L. Adverse Effects of Immune-Checkpoint Inhibitors in Hepatocellular Carcinoma. *OncoTargets therapy*. 2020;13:11725–40.
2. Feitelson M. Parallel Epigenetic and Genetic Changes in the Pathogenesis of Hepatitis Virus-Associated Hepatocellular Carcinoma. *Cancer letters*. 2006;239(1):10–20.
3. Shibata C, Otsuka M, Kishikawa T, Ohno M, Yoshikawa T, Takata A, Koike K. Diagnostic and Therapeutic Application of Noncoding Rnas for Hepatocellular Carcinoma. *World journal of hepatology*. 2015;7(1):1–6.
4. Liu S, Yao X, Zhang D, Sheng J, Wen X, Wang Q, Chen G, Li Z, Du Z, Zhang X. Analysis of Transcription Factor-Related Regulatory Networks Based on Bioinformatics Analysis and Validation in Hepatocellular Carcinoma. *BioMed research international* 2018;2018:1431396.
5. Bruix J, Gores G, Mazzaferro V. Hepatocellular Carcinoma: Clinical Frontiers and Perspectives. *Gut*. 2014;63(5):844–55.
6. Erratum: Global Cancer Statistics. 2018: Globocan Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: a cancer journal for clinicians* 2020;70(4):313.

7. Vogelstein B, Papadopoulos N, Velculescu V, Zhou S, Diaz L, Kinzler K. Cancer Genome Landscapes. *Science* 2013 (New York, N.Y.) ;339(6127):pp. 1546–58.
8. Shannon P, Markiel A, Ozier O, Baliga N, Wang J, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome research*. 2003;13(11):2498–504.
9. Chandrashekar D, Bashel B, Balasubramanya S, Creighton C, Ponce-Rodriguez I, Chakravarthi B, Varambally S. Ualcan: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia* (New York, N.Y.) 2017;19(8):649–658.
10. Gu J, Liu X, Li J, He Y. MicroRNA-144 Inhibits Cell Proliferation, Migration and Invasion in Human Hepatocellular Carcinoma by Targeting Ccnb1. *Cancer cell international*. 2019;19:15.
11. Malumbres M, Barbacid M. Mammalian Cyclin-Dependent Kinases. *Trends Biochem Sci*. 2005;30(11):630–41.
12. Nabetani A, Koujin T, Tsutsumi C, Haraguchi T, Hiraoka YA, Conserved Protein. Nuf2, Is Implicated in Connecting the Centromere to the Spindle During Chromosome Segregation: A Link between the Kinetochore Function and the Spindle Checkpoint. *Chromosoma*. 2001;110(5):322–34.
13. Hu P, Chen X, Sun J, Bie P, Zhang L. Sirna-Mediated Knockdown against Nuf2 Suppresses Pancreatic Cancer Proliferation in Vitro and in Vivo. *Bioscience reports* 2015;35(1).
14. Fu H, Shao L. Silencing of Nuf2 Inhibits Proliferation of Human Osteosarcoma Saos-2 Cells. *Eur Rev Med Pharmacol Sci*. 2016;20(6):1071–9.
15. Zhang W, Mao J, Zhu W, Jain A, Liu K, Brown J, Karpen G. Centromere and Kinetochore Gene Misexpression Predicts Cancer Patient Survival and Response to Radiotherapy and Chemotherapy. *Nature communications*. 2016;7:12619.
16. Hewit K, Sandilands E, Martinez RS, James D, Leung HY, Bryant DM, Shanks E, Markert EK. A Functional Genomics Screen Reveals a Strong Synergistic Effect between Docetaxel and the Mitotic Gene Dlgap5 That Is Mediated by the Androgen Receptor. *Cell Death Dis*. 2018;9(11):1069.
17. Wang Q, Chen Y, Feng H, Zhang B, Wang H. Prognostic and Predictive Value of Hurp in Non–Small Cell Lung Cancer. *Oncol Rep*. 2018;39(4):1682–92.
18. Eissa S, Matboli M, Mansour A, Mohamed S, Awad N, Kotb Y. Evaluation of Urinary Hurp Mrna as a Marker for Detection of Bladder Cancer: Relation to Bilharziasis. *Medical oncology* (Northwood, London, England) 2014;31(2):804.
19. Espinoza I, Sakiyama M, Ma T, Fair L, Zhou X, Hassan M, Zabaleta J, Gomez C. Hypoxia on the Expression of Hepatoma Upregulated Protein in Prostate Cancer Cells. *Frontiers in oncology*. 2016;6:144.
20. Tsou A, Yang C, Huang C, Yu R, Lee Y, Chang C, Chen B, Chung Y, Fann M, Chi C and others. Identification of a Novel Cell Cycle Regulated Gene, Hurp, Overexpressed in Human Hepatocellular Carcinoma. *Oncogene*. 2003;22(2):298–307.
21. Hatfield K, Reikvam H, Bruserud Ø. Identification of a Subset of Patients with Acute Myeloid Leukemia Characterized by Long-Term in Vitro Proliferation and Altered Cell Cycle Regulation of the

- Leukemic Cells. *Expert Opin Ther Targets*. 2014;18(11):1237–51.
22. Cohen Y, Gutwein O, Garach-Jehoshua O, Bar-Haim A, Kornberg A. The Proliferation Arrest of Primary Tumor Cells out-of-Niche Is Associated with Widespread Downregulation of Mitotic and Transcriptional Genes. *Hematology (Amsterdam Netherlands)*. 2014;19(5):286–92.
 23. Zhang Q, Su R, Shan C, Gao C, Wu P. Non-Smc Condensin I, Complex, Subunit G (Ncapg) Is a Novel Mitotic Gene Required for Hepatocellular Cancer Cell Proliferation and Migration. *Oncology research* 2018;26(2):269–276.
 24. Liu W, Liang B, Liu H, Huang Y, Yin X, Zhou F, Yu X, Feng Q, Li E. Zou Z and others. Overexpression of Non-Smc Condensin i Complex Subunit g Serves as a Promising Prognostic Marker and Therapeutic Target for Hepatocellular Carcinoma. *Int J Mol Med*. 2017;40(3):731–8.
 25. Li B, Pu K, Wu X. Identifying Novel Biomarkers in Hepatocellular Carcinoma by Weighted Gene Co-Expression Network Analysis. *Journal of cellular biochemistry* 2019.
 26. Liu Z, Zhang R, Yong Y, Zhang Z, Li C, Chen Z, Bian H. Identification of Crucial Genes Based on Expression Profiles of Hepatocellular Carcinomas by Bioinformatics Analysis. *PeerJ*. 2019;7:e7436.
 27. Zhou L, Du Y, Kong L, Zhang X, Chen Q. Identification of Molecular Target Genes and Key Pathways in Hepatocellular Carcinoma by Bioinformatics Analysis. *OncoTargets therapy*. 2018;11:1861–9.
 28. Vader G, Lens S. The Aurora Kinase Family in Cell Division and Cancer. *Biochim Biophys Acta*. 2008;1786(1):60–72.
 29. Li X, Xu W, Kang W, Wong S, Wang M, Zhou Y, Fang X, Zhang X, Yang H, Wong C and others. Genomic Analysis of Liver Cancer Unveils Novel Driver Genes and Distinct Prognostic Features. *Theranostics* 2018;8(6):1740–1751.
 30. Simon E, Freije C, Farber B, Lalazar G, Darcy D, Honeyman J, Chiaroni-Clarke R, Dill B, Molina H. Bhanot U and others. Transcriptomic Characterization of Fibrolamellar Hepatocellular Carcinoma. *Proc Natl Acad Sci USA*. 2015;112(44):E5916-25.
 31. Chen C, Song G, Xiang J, Zhang H, Zhao S, Zhan Y. Aurka Promotes Cancer Metastasis by Regulating Epithelial-Mesenchymal Transition and Cancer Stem Cell Properties in Hepatocellular Carcinoma. *Biochem Biophys Res Commun*. 2017;486(2):514–20.
 32. Jeng Y, Peng S, Lin C, Hsu H. Overexpression and Amplification of Aurora-a in Hepatocellular Carcinoma. *Clinical cancer research: an official journal of the American Association for Cancer Research*. 2004;10(6):2065–71.
 33. Zhang K, Chen J, Chen D, Huang J, Feng B, Han S, Chen Y, Song H, De W, Zhu Z and others. Aurora-a Promotes Chemoresistance in Hepatocellular Carcinoma by Targeting Nf-Kappab/Microrna-21/Pten Signaling Pathway. *Oncotarget* 2014;5(24):12916-35.
 34. Wang B, Hsu C, Chou C, Lee H, Chiang W, Su C, Tsai H, Yang S, Tang C. Aurkavariations in the Gene: Biomarkers for the Development and Progression of Hepatocellular Carcinoma. *International journal of medical sciences*. 2018;15(2):170–5.
 35. Lu L, Han H, Tian Y, Li W, Zhang J, Feng M, Li Y. Aurora Kinase a Mediates C-Myc's Oncogenic Effects in Hepatocellular Carcinoma. *Mol Carcinog*. 2015;54(11):1467–79.

36. Zhu Q, Luo M, Zhou C, Zhou Z, He Z, Yu X, Zhou S. A Proteomics-Based Investigation on the Anticancer Activity of Alisertib, an Aurora Kinase a Inhibitor, in Hepatocellular Carcinoma Hep3b Cells. *American journal of translational research*. 2017;9(8):3558–72.
37. Chen Q, Xia J, Li W, Shen L, Huang T, Wu P. Examining the Key Genes and Pathways in Hepatocellular Carcinoma Development from Hepatitis B Virus–Positive Cirrhosis. *Mol Med Rep*. 2018;18(6):4940–50.
38. Wang Y, Hou P, Yu H, Wang W, Ji M, Zhao S, Yan S, Sun X, Liu D, Shi B and others. High Prevalence and Mutual Exclusivity of Genetic Alterations in the Phosphatidylinositol-3-Kinase/Akt Pathway in Thyroid Tumors. *J Clin Endocrinol Metab*. 2007;92(6):2387–90.
39. Santarpia L, El-Naggar A, Cote G, Myers J, Sherman S. Phosphatidylinositol 3-Kinase/Akt and Ras/Raf-Mitogen-Activated Protein Kinase Pathway Mutations in Anaplastic Thyroid Cancer. *J Clin Endocrinol Metab*. 2008;93(1):278–84.
40. Watanuki A, Ohwada S, Fukusato T, Makita F, Yamada T, Kikuchi A, Morishita Y. Prognostic Significance of DNA Topoisomerase lialpha Expression in Human Hepatocellular Carcinoma. *Anticancer research*. 2002;22:1113–9.
41. Nakajima T, Yasui K, Zen K, Inagaki Y, Fujii H, Minami M, Tanaka S, Taniwaki M, Itoh Y, Arai S. and others. Activation of B-Myb by E2f1 in Hepatocellular Carcinoma. *Hepatology research: the official journal of the Japan Society of Hepatology*. 2008;38(9):886–95.
42. Chan H, Chu T, Chien H, Sun C, Wang E, Pan H, Kuo H, Hu T, Lai K, Cheng J. and others. Rapid Induction of Orthotopic Hepatocellular Carcinoma in Immune-Competent Rats by Non-Invasive Ultrasound-Guided Cells Implantation. *BMC Gastroenterol*. 2010;10:83.
43. Wang N, Zhu M, Tsao S, Man K, Zhang Z, Feng Y. Mir-23a-Mediated Inhibition of Topoisomerase 1 Expression Potentiates Cell Response to Etoposide in Human Hepatocellular Carcinoma. *Mol Cancer*. 2013;12(1):119.
44. Wong N, Yeo W, Wong W, Wong N, Chan K, Mo F, Koh J, Chan S, Chan A, Lai P and others. Top2a Overexpression in Hepatocellular Carcinoma Correlates with Early Age Onset, Shorter Patients Survival and Chemoresistance. *International journal of cancer*. 2009;124(3):644–52.
45. Fritz P, Cabrera C, Dippon J, Gerteis A, Simon W, Aulitzky W, van der Kuip H. C-ErbB2 and Topoisomerase lialpha Protein Expression Independently Predict Poor Survival in Primary Human Breast Cancer: A Retrospective Study. *Breast cancer research: BCR*. 2005;7(3):R374-84.
46. Panvichian R, Tantiwettrueangdet A, Angkathunyakul N, Leelaudomlapi S. Top2a Amplification and Overexpression in Hepatocellular Carcinoma Tissues. *BioMed research international* 2015;2015:381602.
47. Dingemans A, Witlox M, Stallaert R, van der Valk P, Postmus P, Giaccone G. Expression of DNA Topoisomerase lialpha and Topoisomerase libeta Genes Predicts Survival and Response to Chemotherapy in Patients with Small Cell Lung Cancer. *Clinical cancer research: an official journal of the American Association for Cancer Research*. 1999;5(8):2048–58.

48. Kozovska Z, Gabrisova V, Kucerova L. Colon Cancer: Cancer Stem Cells Markers, Drug Resistance and Treatment. *Biomedicine pharmacotherapy = Biomedecine pharmacotherapie*. 2014;68(8):911–6.
49. Costa M, Hansen C, Holden J, Guinee D. Topoisomerase II Alpha: Prognostic Predictor and Cell Cycle Marker in Surface Epithelial Neoplasms of the Ovary and Peritoneum. *International journal of gynecological pathology: official journal of the International Society of Gynecological Pathologists*. 2000;19(3):248–57.
50. Gordon C, Gulzar Z, Brooks J. Nusap1 Expression Is Upregulated by Loss of Rb1 in Prostate Cancer Cells. *Prostate*. 2015;75(5):517–26.
51. Raemaekers T, Ribbeck K, Beaudouin J, Annaert W, Van Camp M, Stockmans I, Smets N, Bouillon R, Ellenberg J, Carmeliet G. Nusap, a Novel Microtubule-Associated Protein Involved in Mitotic Spindle Organization. *J Cell Biol*. 2003;162(6):1017–29.
52. Gulzar Z, McKenney J, Brooks J. Increased Expression of Nusap in Recurrent Prostate Cancer Is Mediated by E2f1. *Oncogene*. 2013;32(1):70–7.
53. Kokkinakis D, Liu X, Neuner R. Modulation of Cell Cycle and Gene Expression in Pancreatic Tumor Cell Lines by Methionine Deprivation (Methionine Stress): Implications to the Therapy of Pancreatic Adenocarcinoma. *Mol Cancer Ther*. 2005;4(9):1338–48.
54. Zhang X, Pan Y, Fu H, Zhang J. Nucleolar and Spindle Associated Protein 1 (Nusap1) Inhibits Cell Proliferation and Enhances Susceptibility to Epirubicin in Invasive Breast Cancer Cells by Regulating Cyclin D Kinase (Cdk1) and Dlgap5 Expression. *Medical science monitor: international medical journal of experimental clinical research*. 2018;24:8553–64.
55. Roy S, Hooiveld G, Seehawer M, Caruso S, Heinzmann F, Schneider A, Frank A, Cardenas D, Sonntag R, Luedde M and others. MicroRNA 193a-5p Regulates Levels of Nucleolar- and Spindle-Associated Protein 1 to Suppress Hepatocarcinogenesis. *Gastroenterology*. 2018;155(6):1951–66.e26.

Figures

Figure 1

A: Wayne figure, B: Protein interaction network, C: Hub gene interaction network.

Figure 2

A: Pathway analysis of 84 differential gene collections, B: Pathway analysis of 10 hub genes gathering.

Figure 3

The expression of each gene(CCNB1, CCNA2, NCAPG, AURKA, ASPM, TOP2A, DLGAP5, NUSAP1, NUF2 and RACGAP1) was different in tumor tissues and normal tissues.

Figure 4

Survival analysis of each gene (CCNB1, CCNA2, NCAPG, AURKA, ASPM, TOP2A, DLGAP5, NUSAP1, NUF2 and RACGAP1).