European Review for Medical and Pharmacological Sciences 2015; 19: 2054-2061

Bioinformatics analysis of gene expression profiles in hepatocellular carcinoma

H. SHANGGUAN[1], S.-Y. TAN[2], J.-R. ZHANG[3]

[1]Department of Oncology, Foshan Hospital, Southern Medical University, Foshan, Guangdong, P.R. China
[2]Department of Liver Disease, Foshan Hospital, Southern Medical University, Foshan, Guangdong, P.R. China
[3]Oncology Center, Zhujiang Hospital, Southern Medical University, Guangzhou, Guangdong, P.R. China

Abstract. – OBJECTIVE: The aim of this study is to identify the gene expression profile specific to Hepatocellular Carcinoma (HCC) by comparing the different expression profiles in cirrhosis, dysplastic nodule (DN) and HCC tissues.

MATERIALS AND METHODS: The microarray data were downloaded from Gene Expression Omnibus (GEO) repository, involving 39 samples of normal liver tissues, 33 samples of cirrhosis, 17 samples of DNs and 286 samples of HCCs of different stages. Differential Expressed Genes (DEGs) of cirrhosis, DN and HCC liver tissues were analyzed by BRB-ArrayTool software; besides, the Gene Ontology (GO) analysis, Kyoto encyclopedia of Genes and Genomes (KEGG) and Biocarta pathway enrichment analysis were also performed. A protein-protein interaction (PPI) network was then constructed by STRING software using the genes in significantly different pathways. The resulting network was analyzed by Cytoscape software with CentiScaPe plugin to calculate the topological characteristics of the network and its individual node. Key genes were screened according to betweenness and degree of nodes.

RESULTS: few overlaps occurred in the GO categories of DEGs and in the gene sets from pathway analysis between HCCs, cirrhosis and DNs. DEGs in abnormal tissues were shown to be enriched in 29 KEGG pathways and 18 Biocarta pathways; and 43 key genes were identified to be involved in the maintenance of PPI network. In addition, the gene expression profiles were significantly different among cirrhosis, DN and HCC tissues.

CONCLUSIONS: The bioinformatic analysis of GEO datasets of HCC identified the functional gene sets associated with the genesis and development of HCC, and the key genes that were playing important roles in the maintenance of the molecular network for biological function specific to HCC. It provides the insights for more precise understandings of pathogenic mechanism, which will further expand the study on biomarker and targeted therapy of HCC.

Key Words:
Hepatocellular carcinoma, Expression profile, Key gene, Bioinformatics.

Introduction

Hepatocellular Carcinoma (HCC) is the third leading cause of cancer-related death in the worldwide area with increasing incidence[1]. The occurrence of HCC is associated with multiple risk factors, such as hepatitis virus infections and aflatoxin exposure. The progression of most HCCs is a stepwise process, proceeding from chronic hepatitis, cirrhosis and dysplastic nodules (DN) to HCC. A number of genes were found to aberrantly expressed in the tissue of HCC. In the present study, with the aim of discovering the key genes that lead to the aberrant functions of HCC cells, gene expression profiles of cirrhosis, DN and HCC were compared with those of normal liver tissue using microarray analysis to identify differentially expressed genes (DEGs). Subsequently, the pathway analysis was performed to screen specific aberrant signaling pathways in HCC, and thereby to identify the key nodes involved in the maintenance of these pathways, which will further provide the novel therapeutic strategies for HCC via targeted intervention of these genes.

Materials and Methods

Microarray Data

The gene dataset was retrieved from Gene Expression Omnibus (GEO) repository of National Center for Biotechnology Information (NCBI) of National Institute of Health (NIH) of USA. The

Corresponding Author: Jiren Zhang, MD; e-mail: zhangjiren@126.com

inclusion criteria of dataset are listed as follows: (1) the gene expression data series (GSE) of normal liver tissue, cirrhosis, DN and HCC. Microarrays of normal liver tissue should not include the peritumoral liver tissue and the tissue of hepatitis and exclude the microarrays of experimental cell lines; (2) the cross species microarrays were excluded to avoid the effect on the analysis accuracy. As a result, the dataset under annotation of GPL570 ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array) was included in the present study, containing the expression profiles of 47,000 transcripts in human genomes.

Microarray Analysis

The microarray analysis was performed by BRB-Array Tools version 4.4.0 - Beta_1[2]. Gene expression profiles of cirrhosis, DN and HCC were compared with those of normal liver tissue to identify DEGs. The analysis included the following procedures: (1) CEL files of microarrays were imported into Microsoft EXCEL using MAS5.0 algorithm; (2) probes with hybridization signal intensity < 10 were filtered; (3) probe signal values were then converted to log2 value; (4) data were normalized by the Median Normalization module (the system automatically calculated the median log intensity as reference chip, then calculated the differences in log intensity between probes and reference chip, and finally relative mRNA expression level was obtained by subtracting the median differences from the log intensity of each probe); (5) probes which failed to meet the following criteria were filtered: at least 1.5 fold of change occured in the median value of the probe (two-way) and this change was observed in ≥ 20% of samples. A number of samples with missing gene expression data didn't exceed 50%; (6) the affymetrix platform was used for dataset annotation and gene annotation of the probes. The microarray data were analyzed by the univariate t test at a nominal significance level of 0.001; (7) the identified DEGs were analyzed by the Gene ontology (GO) analysis, KEGG and Biocarta pathway analysis. Significantly enriched gene sets were identified. Differences between gene sets were analyzed by the LS/KS permutation test and Efron-Tibshirani's GSA maxmean test with a significance threshold of 0.005; (8) the mean expression ratios of individual genes in each pathway of HCC tissue and normal liver tissue were obtained, and the pathways with mean expression ratio >1 were considered as the pathways with globally increased expression.

Construction of Protein-protein Interaction (PPI) Network

DEGs identified by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Biocarta pathway analyses were uploaded to Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) version 9.1 online tool (http://string-db.org/) in order to construct PPI network of proteins encoded by the DEGs[3]. The STRING software, jointly developed and operated by EMBL, SIB and UZH, is a database containing all known and predicted protein interactions. The interactions include direct (physical) and indirect (functional) associations as derived from four sources, including literature reported protein interactions, genome analysis and prediction, high-throughput experiments and co-expression studies.

Network Visualization and Screening of Key Genes

The PPI networks obtained from the analysis with STRING 9.1 software were imported into Cytoscape software version 3.1.1 for further visual processing[4]. In the meantime, topological characteristics of PPI network and each node were analyzed by CentiScaPe 2.1 pulgin to screen for key nodes. The Cytoscape software enables the integration of various molecular information including biological network, gene expression and genotype in a visual environment; besides, it can directly connect networks with the functional annotation database[5]. Nodes in a network represent genes, proteins or molecules, while connections indicate the interactions between these molecules. The degree in CentiScaPe (node degree) is the total number of edges connected a node. Betweenness of a node is defined as the ratio of the number of shortest paths passing through a node to the total number of paths passing through the node. A higher degree of a node indicates that more genes interact with the node, and a greater value of betweeness of a node represents a greater impact of this gene on the regulation of the entire network. The so-called key genes refer to those genes whose alterations can induce changes of expression of a number of genes downstream of the network. These genes play important roles in maintaining the stability of network. The knockout of these genes may result in the crash of the entire network. Nodes with higher degree and higher betweeness tend to be more essential in the entire network. Functional alterations of these nodes may be crucial to the entire network. Hence, key

genes were screed in accordance of the values of degree and betweeness of the nodes. In the present study, nodes with both degree and betweeness values ≥ mean +1SD, respectively, were considered as key nodes.

Results

Datasets Retrieved

A total of 11 datasets were retrieved from GEO repository and further subjected for the data analysis according to the inclusion criteria, including GSE6222, GSE6764, GSE9843, GSE11045, GSE13471, GSE17548, GSE19665, GSE23343, GSE24042, GSE29721 and GSE45436. These datasets included data from 39 samples of normal liver tissue, 33 samples of cirrhosis, 17 samples of DNs and 286 samples of HCCs at different stages. During the comparison of expression profiles between cirrhosis and normal liver, 19503 genes have been screened, and 7141 aberrantly expressed genes were identified. A total of 16854 genes have been screened when the expression profiles were compared between DN and normal liver, and 5416 aberrantly expressed genes were obtained. When the gene profile of HCC was compared with that of the normal liver, 24384 genes have been screened and 10512 genes have been identified. No overlap of DEG sets was observed between three groups through GO analysis as well as KEGG and Biocarta pathway analysis on DEGs, indicating that gene expression profiles were significantly different between three groups of samples (Table I).

KEGG-identified pathways of HCC group mainly included the following pathways: fatty acid synthesis and metabolism, caffeine metabolism, amino acid metabolism, retinol metabolism, DNA replication, one-carbon metabolism, metabolism of cytochrome p450 enzymes, glycolysis/gluconeogenesis, bicarbonate reabsorption in the proximal tubule, complement and coagulation cascade. The study of HCC tissue revealed several increased expressed pathways, including hsa00010 (glycolysis/gluconeogenesis pathway), hsa00471 (D-glutamine and glutamic acid metabolic pathway), hsa00670 (one-carbon metabolism pathway of folate), hsa00910 (nitrogen metabolism pathway), hsa03030 (DNA replication pathway), hsa04964 (proximal tubule bicarbonate reclamation), hsa04966 (collecting duct acid secretion) and hsa05410 (hypertrophic cardiomyopathy) (Table II).

The following highly expressed enriched pathways were identified through Biocarta pathway analysis, including h_vdrPathway (control of gene expression by vitamin D receptor), h_g2Pathway (cell cycle: G2 / M checkpoint), h_ptc1Pathway (Sonic Hedgehog, Ptc1 regulates cell cycle), h_cdc25Pathway (cdc25 and chk1 regulatory pathway in response to DNA damage), h_antisensePathway (RNA polymerase III transcription), h_atrbrcaPathway (Role of BRCA1, BRCA2 and ATR in Cancer Susceptibility), h_mcmPathway (CDK regulation of DNA replication), h_stathminPathway (Stathmin and breast cancer resistance to antimicrotubule agents), h_rbPathway (RB tumor suppressor /checkpoint signaling in response to

Table I. GO analysis of DEGs in cirrhosis, DN and HCC tissues.

	GO categories of DEGs
Cirrhosis group	GO:0002577, GO:0002579, GO:0010712, GO:0018149, GO:0021859, GO:0022028, GO:0032594, GO:0032595, GO:0032596, GO:0032599, GO:0032651, GO:0032652, GO:0032907, GO:0032910, GO:0032965, GO:0033606, GO:0035860, GO:0044246, GO:0044253, GO:0045112, GO:0045113, GO:0060601, GO:0060737, GO:0071356, GO:0072139, GO:0072182, GO:0072203, GO:0072215, GO:0090183, GO:2000696, GO:2000698
DN group	GO:0002431, GO:0006907, GO:0009445, GO:0030238, GO:0030520, GO:0030850, GO:0032594, GO:0046546, GO:0046661, GO:0046686, GO:0060330, GO:0060334, GO:0060512, GO:0060736, GO:0060740, GO:0072135, GO:0072183, GO:0090185, GO:2000697
HCC group	GO:0000083, GO:0000098, GO:0003014, GO:0006270, GO:0007052, GO:0007098, GO:0008608, GO:0009064, GO:0009069, GO:0009071, GO:0009072, GO:0009081, GO:0009083, GO:0009448, GO:0009698, GO:0022616, GO:0030261, GO:0032508, GO:0042737, GO:0046487, GO:0046950, GO:0050000, GO:0050667, GO:0051297, GO:0051303, GO:0051310, GO:0051983, GO:0060004, GO:0090329

Table II. KEGG pathway analysis of DEGs in cirrhosis, DN and HCC tissues.

	KEGG pathways related to DEGs
Cirrhosis group	hsa00120, hsa00280, hsa00532, hsa04080, hsa04122, hsa04330, hsa04512, hsa04640, hsa04672, hsa04940, hsa04970, hsa04971, hsa04972, hsa04974, hsa05140, hsa05310, hsa05320, hsa05330, hsa05332, hsa05340, hsa05410, hsa05412, hsa05414
DN group	hsa00561, hsa00603, hsa03320, hsa04080, hsa04115, hsa04610, hsa04640, hsa04672, hsa04920, hsa04940, hsa05100, hsa05130, hsa05131, hsa05140, hsa05150, hsa05216, hsa05222, hsa05320, hsa05322, hsa05323, hsa05330, hsa05332, hsa05416
HCC group	hsa00010, hsa00061, hsa00071, hsa00072, hsa00232, hsa00250, hsa00260, hsa00280, hsa00330, hsa00350, hsa00380, hsa00410, hsa00471, hsa00590, hsa00591, hsa00630, hsa00640, hsa00650, hsa00670, hsa00830, hsa00910, hsa00920, hsa00980, hsa00983, hsa03030, hsa04610, hsa04964, hsa04966, hsa05410

DNA damage pathway), h_smPathway (spliceosome assembly), h_ck1Pathway (regulation of Ck1/cdk5 by type 1 glutamate receptors), h_rac1Pathway (Rac 1 cell motility signaling pathway), h_EfpPathway (Estrogen-responsive protein Efp controls cell cycle and breast tumors growth), h_cellcyclePathway (cyclin and cell cycle regulation). Pathways with low expression included h_nuclearRsPathway (nuclear receptors in lipid metabolism and toxicity), h_lectinPathway (lectin induced omplement pathway), h_compPathway (complement pathway), h_ghrelinPathway (ghrelin and the regulation of food intake and energy balance) (Table III).

DEGs of HCC identified through pathway enrichment analysis in the course of KEGG and Biocarta analysis were imported into STRING software to construct PPI network. As a result, a total of 680 nodes were shown to be involved in network construction with 43 key genes identified, including ATIC, CAD, CAT, CCND1, CDK1, CDK2, CREBBP, CYP2A6, CYP2B6, CYP2C19, CYP3A4, CYP3A5, DHFR, ESR1, F2, FTCD, GAPDH, GOT1, GOT2, GSTA3, GSTA4, HADH, IGF1, KNG1, MDH1, MDH2, PCNA , PKLR, PKM2, PLG, POLA1, POLD1, POLE, PPARA, PPARG, PTGS2, RFC4, SERPINC1, SERPINE1, SHMT2, TGFB1, TP53, TYMS. Among these genes, several driver genes involved in multiple cancers were identified, including ATIC, CCND1, CREBBP, FTCD, MDH2, PPARG and TP53 on the basis of data in NCG4.0 database[6].

Table III. Biocarta pathway analysis of DEGs in cirrhosis, DN and HCC tissues.

	Biocarta pathways related to DEGs
Cirrhosis group	h_ifngPathway, h_ucalpainPathway, h_slrpPathway, h_lymphocytePathway, h_npp1Pathway, h_CSKPathway, h_tcraPathway, h_tcytotoxicPathway, h_nktPathway, h_monocytePathway, h_thelperPathway, h_mhcPathway, h_calcineurinPathway, h_lympathway, h_cftrPathway, h_il22bppathway, h_PDZsPathway, h_inflamPathway, h_TPOPathway, h_hSWI-SNFpathway, h_neutrophilPathway, h_il6Pathway, h_plateletAppPathway, h_cell2cellPathway, h_pdgfPathway, h_il17Pathway h_reckPathway, h_il18Pathway, h_gpcrPathway, h_vipPathway, h_mef2dPathway, h_carm1Pathway, h_glycolysisPathway, h_stathminPathway
DN group	h_glycolysisPathway, h_ifngPathway, h_il18Pathway, h_mCalpainPathway, h_cftrPathway, h_mhcPathway, h_il22bppathway, h_egfr_smrtePathway, h_npp1Pathway, h_ucalpainPathway, h_th1th2Pathway, h_mta3Pathway, h_lymphocytePathway, h_vitCBPathway, h_shhPathway, h_ifnaPathway, h_rarrxrPathway, h_il10Pathway, h_monocytePathway, h_ctcfPathway, h_biopeptidesPathway, h_cremPathway, h_gleevecpathway, h_ecmPathway, h_ace2Pathway, h_nkcellsPathway, h_fasPathway, h_IL12Pathway, h_tnfr1Pathway, h_compPathway
HCC group	h_nuclearRsPathway, h_vdrPathway, h_lectinPathway, h_g2Pathway, h_ptc1Pathway, h_cdc25Pathway, h_antisensePathway, h_compPathway, h_atrbrcaPathway, h_mcmPathway, h_stathminPathway, h_rbPathway, h_smPathway, h_ck1Pathway, h_rac1Pathway, h_EfpPathway, h_ghrelinPathway, h_cellcyclePathway

Discussion

A progression from cirrhosis, DN to HCC is a typical pathway of HCC development. In the present study, the gene expression profiles were compared among these three pathological conditions and a number of aberrantly expressed genes were identified.

Fewer genes and proteins act alone. Conversely, they usually interact through a complex network and affect the functions of biological system collaboratively. The impact of multiple genes interacting through a signaling pathway on cellular functions is more significant than that of a single gene; furthermore, the influence of genes on cellular functions is achieved through alterations in signaling pathways. Therefore, in the present study, module analysis was performed to identify genes in the pathways of DEG sets, thereby narrowing the scope of genetic screening; subsequently, the PPI network analysis of encoded genes was performed to identify key nodes that were crucial in maintaining the function of the entire network. These genes have been shown to play important roles in the regulation of these pathways and the maintenance of aberrant functions of HCC cells.

Also in the present study, GO categories of DEGs and pathways were compared among three pathological conditions and no substantial overlap was observed, which indicated that alterations in signalling pathways in these three pathological conditions were not progressively accumulated; however, each pathway collection represented completely different molecular function status under different pathological conditions. As a result, the gene sets of HCC signaling pathway included in PPI network analysis could represent the global molecular status specific to HCC.

The GO analysis and the KEGG pathway analysis have identified more amino acid metabolism pathways in significant DEG set. Genes with elevated expression levels have been shown to be mainly involved in DNA synthesis and repair, as well as in energy metabolism, which is associated with the vigorous growth of cancer cells. Biocarta pathways were enriched in cell cycle regulation as well as DNA damage and repair pathways. Previous studies have shown that vitamin D controlled gene expression signaling pathway, which associated with the development of multiple tumors. This pathway can influence tumor cell growth by affecting the functions of ß-catenin pathway and MAPK singling pathway, which influences the functions of insulin-like growth factor (IGF), transforming growth factor beta (TGF-ß) and epidermal growth factor receptor (EGFR)[7,8]. The cell cycle signaling pathway regulated by Sonic Hedgehog receptor, Ptc1, is associated with the development of gastric cancer, pancreatic cancer and HCC, and other digestive tract tumours[9-13]. Stathmin is over-expressed in primary liver cancer. It is also involved in the development and progression of HCC possibly by promoting cell proliferation and suppressing cell apoptosis through regulation of the expressions of tumor proliferation related genes[14-17]. Stathmin expression has also been shown to be elevated in non-small cell lung cancer, oral squamous-cell carcinoma and ovarian cancer[18-20].

Forty-three nodes identified in this study are involved in different pathways and locate at multiple node positions in the network. Removal of these nodes can lead to the collapse of the entire network. Among these genes, seven were identified as driver genes of multiple tumors. The 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC) locates on chromosome 2q35. The encoded ATIC protein is a bi-functional enzyme that exhibits catalytic activities of aminoimidazolecarboxamide ribonucleotide transformylase and inosine monophosphate cyclohydrolase. It is involved in the *de novo* purine nucleotide synthesis pathway, catalyzing the formylation of 5-amino-4-imidazole carboxamide ribonucleotide to generate 5-formamidoimidazole-4-carboxamide nucleotides, which is then catalyzed to IMP through dehydration and cyclization and, thereby, leading to the synthesis of adenine nucleotide (AMP) and guanine nucleotide (GMP). ATIC is a driver gene of lymphoma, which is associated with the development of colorectal cancer and ovarian cancer[21-23]. Cell cyclin D1 (CCND1) gene locates on chromosome 11q13 and plays a key role in cell cycle G1/S control. It has been shown to be overexpressed in multiple tumors and is the driver gene for breast cancer, lung cancer, leukemia and myeloma. CCND1 has been shown to be associated with a number of signaling pathways, including p53, PI3K-Akt, AMPK, Wnt, JAk-STAT, Ras, Rap1, MAPK, ErbB signaling pathways and its mutation can increase the risk of HCC[24]. CREB binding protein

(CREBBP) gene locates on chromosome 16p13.3. The encoded protein exhibits histone acetyl- transferase activity and can promote the binding of transcription factors to DNA, which is beneficial to the gene transcription; therefore, this gene is involved in a wide range of physiological process, such as embryonic development and growth regulation. Previous studies have shown that this gene is involved in multiple tumor-related signaling pathway, such as MAPK signaling pathway and is a driver gene in leukemia, lymphoma and bladder cancer[25]. Formiminotransferase cyclodeaminase (FTCD) gene locates on chromosome 21q22.3 and exhibits transferase and deaminase activities. FTCD is involved in the transportation of one-carbon units from formimidoylglutamate to folic acid, and also a driver gene for lymphoma[26]. The results of this study showed that this gene was expressed at low level in HCC tissue. Malate dehydrogenase 2 (MDH2) locates on chromosome 12q14.3-q15. It assists in catalyzing the oxidation of malate to oxaloacetate in the citric acid cycle and is the driver gene of colorectal cancer, glioma, sarcoma and lung cancer. Our results showed the low expression of this gene in HCC tissue. Peroxisome proliferator-activated receptor gamma (PPARG) locates on chromosome 3p25. A variety of genes involved in fatty acid transportation and metabolism are regulated by PPARG on transcriptional level, such as adipocyte fatty acid binding protein (AFABP), fatty acid transport protein (FATP) and lipoprotein lipase (LPL). PPARG can enhance the expressions of fatty acid transport proteins and fatty acid transferase, and stimulate cellular uptake of fatty acids and the transformation to fatty acyl coenzyme A. PPARG is the driver gene of thyroid cancer. Tumor protein p53 (TP53), locating on chromosome 17p13.1, is a tumor suppressor gene whose expression can induce the stagnation of cell growth or apoptosis. TP53 mutation occurs in a wide range of tumors such as HCC[27-30].

Conclusions

The microarray of HCC included in this study contains HCC samples originating from a variety of causes; therefore, the signaling pathways and PPI networks obtained can represent common molecular functional changes in HCCs of various etiologies. These signaling pathways we identified are different from those in cirrhosis and DN. The PPI network constructed by the genes obtained from these signaling pathways can represent molecular network specific to HCC, and the nodes in the PPI network are of paramount importance to the stability and functional maintenance of the entire network. Novel therapeutic strategies against aforementioned key nodes of the network can shed the light on the future targeted therapy of HCC.

Acknowledgements

The present study has adopted the BRB-ArrayTools developed by Dr. Richard Simon and BRB-ArrayTools development team.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.
2) Simon R, Lam A, Li MC, Ngan M, Menenzes S, Zhao Y. Analysis of gene expression data using BRB-ArrayTools. Cancer Inform 2007; 3: 11-17.
3) Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Minguez P, Bork P, von Mering C, Jensen LJ. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 2013; 41: D808-15.
4) Saito R, Smoot ME, Ono K, Ruscheinski J, Wang PL, Lotia S, Pico AR, Bader GD, Ideker T. A travel guide to Cytoscape plugins. Nat Methods 2012; 9: 1069-1076.
5) Scardoni G, Petterlini M, Laudanna C. Analyzing biological network parameters with CentiScaPe. Bioinformatics 2009; 25: 2857-2859.
6) An O, Pendino V, D'Antonio M, Ratti E, Gentilini M, Ciccarelli FD. NCG 4.0: the network of cancer genes in the era of massive mutational screenings of cancer genomes. Database (Oxford) 2014; 2014: bau015.
7) Pereira F, Barbachano A, Silva J, Bonilla F, Campbell MJ, Munoz A, Larriba MJ. KDM6B/JMJD3 histone demethylase is induced by vitamin D and modulates its effects in colon cancer cells. Hum Mol Genet 2011; 20: 4655-4665.
8) Han S, Li T, Ellis E, Strom S, Chiang JY. A novel bile acid-activated vitamin D receptor signaling in human hepatocytes. Mol Endocrinol 2010; 24: 1151-1164.

9) Ohta M, Tateishi K, Kanai F, Watabe H, Kondo S, Guleng B, Tanaka Y, Asaoka Y, Jazag A, Imamura J, Ijichi H, Ikenoue T, Sata M, Miyagishi M, Taira K, Tada M, Kawabe T, Omata M. p53-Independent negative regulation of p21/cyclin-dependent kinase-interacting protein 1 by the Sonic hedgehog-glioma-associated oncogene 1 pathway in gastric carcinoma cells. Cancer Res 2005; 65: 10822-10829.

10) Thayer SP, di Magliano MP, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, Qi YP, Gysin S, Fernández-del Castillo C, Yajnik V, Antoniu B, McMahon M, Warshaw AL, Hebrok M. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature 2003; 425: 851-856.

11) Patil MA, Zhang J, Ho C, Cheung ST, Fan ST, Chen X. Hedgehog signaling in human hepatocellular carcinoma. Cancer Biol Ther 2006; 5: 111-117.

12) Huang S, He J, Zhang X, Bian Y, Yang L, Xie G, Zhang K, Tang W, Stelter AA, Wang Q, Zhang H, Xie J. Activation of the hedgehog pathway in human hepatocellular carcinomas. Carcinogenesis 2006; 27: 1334-1340.

13) Berman DM, Karhadkar SS, Maitra A, Montes De Oca R, Gerstenblith MR, Briggs K, Parker AR, Shimada Y, Eshleman JR, Watkins DN, Beachy PA. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. Nature 2003; 425: 846-851.

14) Yuan RH, Jeng YM, Chen HL, Lai PL, Pan HW, Hsieh FJ, Lin CY, Lee PH, Hsu HC. Stathmin overexpression cooperates with p53 mutation and osteopontin overexpression, and is associated with tumour progression, early recurrence, and poor prognosis in hepatocellular carcinoma. J Pathol 2006; 209: 549-558.

15) Li C, Tan YX, Zhou H, Ding SJ, Li SJ, Ma DJ, Man XB, Hong Y, Zhang L, Li L, Xia QC, Wu JR, Wang HY, Zeng R. Proteomic analysis of hepatitis B virus-associated hepatocellular carcinoma: Identification of potential tumor markers. Proteomics 2005; 5: 1125-1139.

16) Chen Y, Lin MC, Yao H, Wang H, Zhang AQ, Yu J, Hui CK, Lau GK, He ML, Sung J, Kung HF. Lentivirus-mediated RNA interference targeting enhancer of zeste homolog 2 inhibits hepatocellular carcinoma growth through down-regulation of stathmin. Hepatology 2007; 46: 200-208.

17) Singer S, Ehemann V, Brauckhoff A, Keith M, Vreden S, Schirmacher P, Breuhahn K. Protumorigenic overexpression of stathmin/Op18 by gain-of-function mutation in p53 in human hepatocarcinogenesis. Hepatology 2007; 46: 759-768.

18) Chen G, Wang H, Gharib TG, Huang CC, Thomas DG, Shedden KA, Kuick R, Taylor JM, Kardia SL, Misek DE, Giordano TJ, Iannettoni MD, Orringer MB, Hanash SM, Beer DG. Overexpression of oncoprotein 18 correlates with poor differentiation in lung adenocarcinomas. Mol Cell Proteomics 2003; 2: 107-116.

19) Kouzu Y, Uzawa K, Koike H, Saito K, Nakashima D, Higo M, Endo Y, Kasamatsu A, Shiiba M, Bukawa H, Yokoe H, Tanzawa H. Overexpression of stathmin in oral squamous-cell carcinoma: correlation with tumour progression and poor prognosis. Br J Cancer 2006; 94: 717-723.

20) Karst AM, Levanon K, Duraisamy S, Liu JF, Hirsch MS, Hecht JL, Drapkin R. Stathmin 1, a marker of PI3K pathway activation and regulator of microtubule dynamics, is expressed in early pelvic serous carcinomas. Gynecol Oncol 2011; 123: 5-12.

21) Trinei M, Lanfrancone L, Campo E, Pulford K, Mason DY, Pelicci PG, Falini B. A new variant anaplastic lymphoma kinase (ALK)-fusion protein (ATIC-ALK) in a case of ALK-positive anaplastic large cell lymphoma. Cancer Res 2000; 60: 793-798.

22) Hazra A, Fuchs CS, Kawasaki T, Kirkner GJ, Hunter DJ, Ogino S. Germline polymorphisms in the one-carbon metabolism pathway and DNA methylation in colorectal cancer. Cancer Causes Control 2010; 21: 331-345.

23) Goode EL, Chenevix-Trench G, Hartmann LC, Fridley BL, Kalli KR, Vierkant RA, Larson MC, White KL, Keeney GL, Oberg TN, Cunningham JM, Beesley J, Johnatty SE, Chen X, Goodman KE, Armasu SM, Rider DN, Sicotte H, Schmidt MM, Elliott EA, Høgdall E, Kjær SK, Fasching PA, Ekici AB, Lambrechts D, Despierre E, Høgdall C, Lundvall L, Karlan BY, Gross J, Brown R, Chien J, Duggan DJ, Tsai YY, Phelan CM, Kelemen LE, Peethambaram PP, Schildkraut JM, Shridhar V, Sutphen R, Couch FJ, Sellers TA; Ovarian Cancer Association Consortium. Assessment of hepatocyte growth factor in ovarian cancer mortality. Cancer Epidemiol Biomarkers Prev 2011; 20: 1638-1648.

24) Hu Z, Zhou Z, Xiong G, Wang Y, Lai Y, Deng L, Yang J. Cyclin D1 G870A polymorphism and the risk of hepatocellular carcinoma in a Chinese population. Tumour Biol 2014; 35: 5607-5612.

25) Inthal A, Zeitlhofer P, Zeginigg M, Morak M, Grausenburger R, Fronkova E, Fahrner B, Mann G, Haas OA, Panzer-Grümayer R. CREBBP HAT domain mutations prevail in relapse cases of high hyperdiploid childhood acute lymphoblastic leukemia. Leukemia 2012; 26: 1797-1803.

26) Love C, Sun Z, Jima D, Li G, Zhang J, Miles R, Richards KL, Dunphy CH, Choi WW, Srivastava G, Lugar PL, Rizzieri DA, Lagoo AS, Bernal-Mizrachi L, Mann KP, Flowers CR, Naresh KN, Evens AM, Chadburn A, Gordon LI, Czader MB, Gill JI, Hsi ED, Greenough A, Moffitt AB, McKinney M, Banerjee A, Grubor V, Levy S, Dunson DB, Dave SS. The genetic landscape of mutations in Burkitt lymphoma. Nat Genet 2012; 44: 1321-1325.

27) Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, Calderaro J, Bioulac-Sage P, Letexier M, Degos F, Clément B, Balabaud C,

Chevet E, Laurent A, Couchy G, Letouzé E, Calvo F, Zucman-Rossi J. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 2012; 44: 694-698.

28) Huang J, Deng Q, Wang Q, Li KY, Dai JH, Li N, Zhu ZD, Zhou B, Liu XY, Liu RF, Fei QL, Chen H, Cai B, Zhou B, Xiao HS, Qin LX, Han ZG. Exome sequencing of hepatitis B virus-associated hepatocellular carcinoma. Nat Genet 2012; 44: 1117-1121.

29) Li M, Zhao H, Zhang X, Wood LD, Anders RA, Choti MA, Pawlik TM, Daniel HD, Kannangai R, Offerhaus GJ, Velculescu VE, Wang L, Zhou S, Vogelstein B, Hruban RH, Papadopoulos N, Cai J, Torbenson MS, Kinzler KW. Inactivating mutations of the chromatin remodeling gene ARID2 in hepatocellular carcinoma. Nat Genet 2011; 43: 828-829.

30) Jin B, Wang W, Du G, Huang GZ, Han LT, Tang ZY, Fan DG, Li J, Zhang SZ. Identifying hub genes and dysregulated pathways in hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 2015; 9: 592-601.