Pathology & Oncology Research (2019) 25:1207–1213
https://doi.org/10.1007/s12253-019-00596-2

ORIGINAL ARTICLE

Identification of the Pathogenic Biomarkers for Hepatocellular Carcinoma Based on RNA-seq Analyses

Wentao Jiang[1] · Li Zhang[1] · Qingjun Guo[1] · Honghai Wang[1] · Ming Ma[1] · Jisan Sun[1] · Chiyi Chen[1]

Received: 19 July 2018 / Accepted: 15 January 2019 / Published online: 24 January 2019

Abstract

The purpose of this study was to explore potential biomarkers in the diagnosis of hepatocellular carcinoma (HCC) based on RNA-seq. The microarray data GSE98269 were downloaded from the GEO database, including the miRNA, mRNA and lncRNA expression profiles of 3 HCC tissues and 3 normal liver tissues from 3 HCC patients. The limma package was used to identify the differentially expressed miRNAs (DEMs) and the differentially expressed lncRNAs in HCC tissues compared with normal liver tissues. Database of DAVID, KEGG PATHWAY and Reactome were used to perform the functional and pathway enrichment. Putative targets for DEMs, and the miRNA-gene pairs were predicted via the miRWalk V2.0 database. The protein-protein pairs of DEGs were screened via String software. The expression features of the differentially expressed lncRNAs were analyzed. The regulated network of DEGs and DEMs were constructed, and related genes and miRNAs were detected in the HCC tissues and normal liver samples with Q-PCR. A total of 678 DEGs, 32 DEMs and 411 differential expressed lncRNAs were identified. The DEGs were enriched in 196 GO terms and 79 pathways. 38 negative regulation miRNA-gene pairs and 1205 protein-protein interactions were screened out, and the regulated network was constructed based on them. *KNG1*, *CDK1*, *EHHADH*, *CYP3A4, hsa-miR-199a-5p* and *hsa-miR-455-3p* might be biomarkers in the occurrence of HCC.

Keywords Hepatocellular carcinoma (HCC) · Early diagnosis · RNA-seq

Introduction

Hepatocellular carcinoma (HCC) is a highly aggressive solid malignancy in the word with the high incidence and case fatality rate, which is the most common primary liver cancer in adults. HCC is the second global cause of cancer-related deaths, and the high global incidence and late presentation of HCC may be responsible for it [1, 2]. HCC affects men more than women. The American Cancer Society estimated that the mortality rate of HCC is fifth most in men (19,610 cases per year, 6% of the total) and ninth in women (9, 310 cases per year, 3% of the total) in America [3]. Most HCC develops in patients with underlying chronic liver disease, and hence professional societies recommend HCC screening in patients with cirrhosis. Most of HCC is diagnosed at an advanced stage, which has greatly limited the application, efficacy and prognosis of curative treatment. Therefore, early diagnosis is a key issue to be able to significantly reduce HCC-related mortality, which is one of the most relevant issues that deserve further efforts from scientific community [4]. The diagnosis of HCC mainly depends on medical imaging, and important advancements have been made in the last year. The evaluation of both asymptomatic patients and those with symptoms of liver disease involves blood testing and imaging evaluation. Despite it, there are several areas that still need further advancements in the diagnosis. Tumor markers could be a useful tool to overcome the theoretical limitations of imaging. The clinical measurements of biomarkers can be carried out in vivo by using imaging modalities like ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI), as well as in vitro by utilizing serum or plasma or other body fluids as specimens [5]. Kiyokawa et al. [6] reported that serum monomeric laminin-γ2 and des-gamma-carboxy prothrombin (DCP) were sensitive for laboratory diagnosis of HCC. Both

Wentao Jiang and Li Zhang contributed equally to this work.

✉ Wentao Jiang
wentaojiang23@163.com

✉ Li Zhang

[1] Department of Liver Transplantation, Key Laboratory for Critical Care Medicine of the Ministry of Health, Tianjin Key Laboratory for Organ Transplantation, Tianjin First Center Hospital, 24 Fukang Road, Nankai District, Tianjin 300192, People's Republic of China

miR-221 and miR-101–1 expression levels were thought to be useful as noninvasive diagnostic biomarkers for early prediction of HCC among HCV patients [7]. However, most tumor markers have shown a low diagnostic accuracy, and some panels for the early diagnosis need further external validation of their efficacy [8–10]. In this study, we comprehensively analyzed the mRNA, miRNA and long non-code RNA expression profiles in HCC tissues compared with normal liver tissues, in order to explore more molecular biomarkers in the diagnosis of HCC.

Materials and Methods

Microarray Data The Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo/) is a National Center for Biotechnology Information (NCBI) database to help users query and download experiments and curated gene expression profiles. Here, the microarray data GSE98269 was downloaded from the GEO database. In this microarray data, the microRNA (miRNA), mRNA and long non-coding RNA (lncRNA) profiles of 3 HCC tissues and 3 normal liver tissues were contained from 3 HCC patients. Moreover, the miRNA data was detected with the platform of Agilent-070156 Human miRNA, and the mRNA and lncRNA data were tested with the Agilent-074348 Human LncRNA v6 4X180K platform.

Data Processing and Differential Expressed Analysis Background correction and standardization for the raw data were carried out with the function package of preprocessCore V1.32.0 (http://www.bioconductor.org/packages/3.2/bioc/html/preprocessCore.html). The probe symbols were converted into gene or miRNA or lncRNA symbols. If multiple probes corresponded to one gene or miRNA or lncRNA, the average expression values of them were considered as the expression value of the gene or miRNA or lncRNA. Afterwards, the differentially expressed genes (DEGs), the differentially expressed miRNAs (DEMs) and the differentially expressed lncRNAs were identified in HCC tissues compared with normal liver tissues with limma V3.32.2 (http://www.bioconductor.org/packages/3.5/bioc/html/limma.html). The threshold criteria of DEMs was |log (fold-change)| > 1 and $P < 0.05$, and it was |log (fold-change)| > 2 and P < 0.05 for DEGs and the differentially expressed lncRNAs.

Functional and Pathways Enrichment Analyses of the DEGs The functional and pathways analyses of DEGs were performed via the Database for Annotation, Visualization and Integrated Discovery (DAVID) V6.8 (http://david.abcc.ncifcrf.gov/), Kyoto Encyclopedia of Genes and Genomes (KEGG) PATHWAY (http://www.genome.jp/kegg), and Reactome(http://www.reactome.org). The gene ontology (GO) terms and pathway terms were selected out with $P < 0.05$.

Targets Prediction for DEMs Potential targets of DEMs, and the miRNA-gene pairs were predicted by >5 bioinformatics algorithms among the 10 algorithms in the miRWalk V2.0 database (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/): miRWalk V2.0 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/mirwalk), miRDB V4.0 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/mirdb), miRanda -rel2010 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/miranada), DIANAmT V4.0 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/diana-microt), PICTAR4 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/pictar4), PICTAR5 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/pictar5), PITA (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/pipa), RNAhybrid V2.1 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/rnahybrid), RNA22 V2 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/rna22) and Targetscan V6.2 (www.ma.uni-heidelberg.de/apps/zmf/mirwalk/targetscan). The negatively regulated miRNA-gene pairs were further screened out.

Construction of Regulated Network The protein-protein pairs of DEGs were identified via String V10.5 (https://string-db.org/) with more than 700 scores. Ultimately, the regulated network was established based on above negatively regulated miRNA-gene pairs and the protein-protein pairs, and visualized by Cytoscape V3.5.1 software (http://www.cytoscape.org/download.php).

Verification of Related Genes and miRNA The HCC tissues and their normal liver tissues were collected from 3 HCC patients accepted by our hospital, and all the patients were provided the informed consent forms. The quantitative real-time PCR (Q-PCR) was conducted to detect the mRNA expression levels of *KNG1*, *CDK1*, *EHHADH*, *CYP3A4, hsa-miR-199a-5p* and *hsa-miR-455-3p* with the ABI Am1005 AgPath-ID™ One-Step RT-PCR Kit (Invitrogen, America) according to the manufacturer's instructions. The reaction conditions were 95 °C for 15 min and 40 cycles of denaturation at 95 °C for 20s, annealing at 57 °C for 20s and extension at 72 °C for 35 s. All the primer sequences were showed in Table 1.

Statistical Analysis SPSS V17.0 (SPSS Inc., Chicago, IL) was used for all statistical analyses, and data were expressed as the mean ± SD. Student's t test was used to compare both two groups, and $P < 0.05$ was considered statistically significant.

Results

DEGs, DEMs and Differential Expressed lncRNAs A total of 678 (203 up- and 475 down-regulated) DEGs, 32 (6 up-

Table 1 The primer sequences of *KNG1*, *CDK1*, *EHHADH*, *CYP3A4, hsa-miR-199a-5p* and *hsa-miR-455-3p*

Genes	Primer	Size
KNG1	5'-GGTTGGCTCTGACACGTTTT-3' 5'-TGGGTAGCCACGGAGAATTT-3'	840 bp
CDK1	5′- ACCTACACTTACCCTTGGCC-3' 5'-TTTTAGGAGCTGGGACTGGG-3'	780 bp
EHHADH	5'-GGGAGGGATAGCGTTAGGAG-3' 5'-TGATGGACATGTGGGTTGGT-3'	1050 bp
CYP3A4	5'-GGGAGGGATAGCGTTAGGAG-3' 5'-TGATGGACATGTGGGTTGGT-3'	980 bp
hsa-miR-199a-5p	5'-CCCAGTGTTCAGACTACCTGTTC-3' 5′-TGAGTGTGTGTGTGAGTGTGT-3'	600 bp
hsa-miR-455-3p	5'-GCAGTCCATGGGCATATACAC-3' 5′-TGAGTGTGTGTGTGAGTGTGT-3'	550 bp
β-actin	5'-ATTATTATTGGTAATGAGCGGTTTC-3' 5'-TTCATAATAAAATTAAATATAATTTCGTA-3'	308 bp

and 26 down-regulated) DEMs and 411 (55 up- and 386 down-regulated) differential expressed lncRNAs were identified in HCC tissues compared with normal liver tissues. The top 20 most significant DEGs, DEMs and differential expressed lncRNAs were showed in Tables 2, 3 and 4, respectively.

The Enriched Gene Ontology (GO) Terms and Pathways The 678 DEGs were enriched in 196 GO terms and 79 pathways. Most GO terms were associated with organic acid metabolism, small molecule metabolism and redox process, and the top 10 most significant GO terms were exhibited in Table 5. Most pathway terms were involved in metabolic pathway, fatty acid degradation pathway and exogenous substance pathway, and the top 10 most significant pathways were showed in Table 6.

The Regulated Network After screening with miRwalk V2.0, 90 miRNA-gene pairs were selected, and among them, 38 pairs were negatively regulated. Furthermore, 1205 high confidence protein-protein pairs of DEGs were obtained with String V10.5. Lastly, the regulated network were constructed based on the 1205 protein-protein pairs and the 38 negatively regulated miRNA-gene pairs, and it was exhibited in Fig. 1. The network mainly were divided into 4 gene clusters, which *KNG1*, *CDK1*, *EHHADH* and *CYP3A4* (dark yellow) were involved in more pairs in each cluster, and the 4 gene clusters were named as *KNG1* gene cluster, *CDK1* gene cluster, *EHHADH* gene cluster and *CYP3A4* gene cluster, respectively. Moreover, *hsa-miR-199a-5p* and *hsa-miR-455-3p* (light yellow) were the only 2 DEMs in the network, which connected *KNG1* gene cluster and *CDK1* gene cluster. Moreover, Q-PCR results showed in Table 7, and the above 4 genes and 2 miRNAs were significantly different in HCC group compared with control group. Besides, *CDK1* was high expressed in HCC tissues, and other 3 genes and the above miRNAs were low expressed.

The Differentially Expressed lncRNAs and the DEGs 39.9% differentially expressed lncRNAs were located in

Table 2 The top 20 most significant DEGs in HCC tissues compared with normal liver tissues

Gene	logFC	P	Gene	logFC	P
SPINK1	10.33707917	0.019556938	FADS6	−2.7946896	0.042166509
ADCY10	−2.674445567	0.019556938	BCKDHB	−2.934083733	0.042166509
LCAT	−3.419683667	0.019556938	CPEB3	−3.074063617	0.042166509
CNDP1	−6.5707004	0.019556938	GSTZ1	−3.459545217	0.042166509
ASPA	−3.620665783	0.040210927	LRAT	−3.529013383	0.042166509
B9D1	2.0349211	0.042166509	CNGA1	−3.915575017	0.042166509
IGFALS	−2.218246	0.042166509	C14orf180	−4.724349033	0.042166509
LIFR	−2.365803367	0.042166509	MAPK12	2.4904995	0.04286853
BDH2	−2.371884167	0.042166509	KCNAB2	2.026920267	0.04286853
MPDZ	−2.734688	0.042166509	BCO2	−2.119811667	0.04286853

DEGs, differentially expressed genes; HCC, hepatocellular carcinoma

Table 3 The top 20 most significant DEMs in HCC tissues compared with normal liver tissues

Gene	logFC	P	Gene	logFC	P
hsa-miR-34b-5p	1.183489103	0.174452078	hsa-miR-30a-3p	−1.071341838	0.409247261
hsa-miR-7114-5p	−1.184010703	0.174452078	hsa-miR-378a-5p	−1.075791095	0.409247261
hsa-miR-130a-3p	−1.50908556	0.174452078	hsa-miR-30a-5p	−1.16401174	0.409247261
hsa-miR-5100	−1.369161432	0.212064788	hsa-miR-30e-3p	−1.410867747	0.409247261
hsa-miR-378a-3p	−2.262145155	0.212064788	hsa-miR-424-5p	−1.513592505	0.409247261
hsa-miR-378i	−2.393404937	0.212064788	hsa-miR-455-3p	−1.648488132	0.409247261
hsa-miR-224-5p	3.449135838	0.246421435	hsa-miR-30e-5p	−1.095039697	0.539249478
hsa-miR-34a-5p	1.712028157	0.253974053	hsa-miR-1260a	−1.532881215	0.539249478
hsa-miR-144-3p	−2.022964177	0.253974053	hsa-miR-21–5p	1.722869662	0.620679218
hsa-miR-378d	−1.148090582	0.347153208	hsa-miR-122–3p	−3.979028223	0.665561342

DEMs, differentially expressed microRNA; HCC, hepatocellular carcinoma

intergenic regions, and about 50% of them were on chromosome 1, and most of them were under 1000 bp. 70 pairs were identified between the differentially expressed lncRNAs and the DEGs, including 62 pairs with same expressed trend and 8 pairs with opposite expressed trend.

Discussion

In our study, *KNG1*, *CDK1*, *EHHADH* and *CYP3A4* were selected out after differential expression analysis and regulated network construction, and they were further verified to abnormally express in HCC tissues (Fig. 1 and Table 7). Kininogen-1 (KNG1), encoded by the *KNG1* gene, is a constituent of the blood coagulation system as well as the kinin-kallikrein system, and it is the precursor protein to high-molecular-weight kininogen (HMWK), low-molecular-weight kininogen (LMWK), and bradykinin [11, 12]. Recently, the KNG1 protein was identified as a potential marker of early colorectal cancer stages by proteomics and immunohistochemistry [13]. A study found that aberrant ceRNA-mediated regulation of *KNG1* contributed to glioblastoma-induced angiogenesis, which provided potential targets for the development of novel therapeutic strategies for glioblastoma [14]. HCC frequently includes abnormalities in cell cycle regulators, including up-regulated cyclin-dependent kinase (CDKs) activities due to loss or low expression of CDK inhibitors [15]. Cyclin-dependent kinase 1 (CDK1), a member of CDKs and encoded by *CDK1*, is a key player in cell cycle regulation and an anti-cancer target [16]. Zhao et al. [17] confirmed that CDK1 activity was detected in primary HCC tissue samples but not in healthy paraneoplastic tissues, and CDK1 and apoptin might be a novel cellular signaling pathway to modulate apoptosis in HCC. Zhang et al. [18] further showed that *miR-582-5p* regulated the progression of HCC through directly inhibiting the expression of CDK1. *EHHADH* is a human gene that

Table 4 The top 20 most significant differentially expressed lncRNAs in HCC tissues compared with normal liver tissues

Gene	logFC	P	Gene	logFC	P
lnc-AC009237.1–4	−2.8458958	0.031392814	lnc-ADRA1B-1	−3.3573383	0.04254741
lnc-DPYSL2–3	−2.764150083	0.031392814	lnc-HSD11B1–1	−2.907965233	0.04254741
lnc-JAKMIP2–1	10.42126862	0.031392814	lnc-PLIN2–1	−3.94011835	0.045460398
lnc-TTK-6	−3.243900933	0.031392814	lnc-AKAP5–1	−2.599524017	0.047382052
lnc-TXNL4B-1	−3.2891669	0.031392814	lnc-CCDC125–6	−2.909481467	0.047382052
lnc-H2AFB2–2	−2.714204417	0.0328605	lnc-FAM35B-4	−2.0398422	0.047382052
lnc-TNKS-2	−2.828453483	0.033356993	lnc-PPYR1–5	−2.0001237	0.047382052
lnc-TTK-7	−3.699033267	0.033356993	RP11-250B2.6	−2.633541	0.047382052
lnc-LY86–7	−2.711439267	0.036380664	LOC153684	2.04617028	0.051964573
AC104809.2	−4.518514425	0.039811755	lnc-ZNF22–3	2.001438267	0.053512742

HCC, hepatocellular carcinoma

Table 5 The top 10 most significant enriched GO terms of DEGs

Category	Term	Count	P
BP	GO:0006082~organic acid metabolic process	141	2.18E-51
BP	GO:0044281~small molecule metabolic process	193	4.01E-41
BP	GO:0055114~oxidation-reduction process	126	1.43E-36
BP	GO:0044712~single-organism catabolic process	103	4.02E-28
BP	GO:0006629~lipid metabolic process	123	1.97E-22
CC	GO:1903561~extracellular vesicle	188	7.12E-19
CC	GO:0043230~extracellular organelle	188	7.40E-19
CC	GO:0070062~extracellular exosome	186	2.20E-18
BP	GO:0044711~single-organism biosynthetic process	114	5.98E-18
BP	GO:0006520~cellular amino acid metabolic process	44	9.81E-18

GO, gene ontology; DEGs, differentially expressed genes; BP, biological process; CC, cellular component

encodes for the bifunctional enzyme enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase (EHHADH), which is one of the four enzymes of the peroxisomal beta-oxidation pathway. Mutations of *EHHADH* are a cause of peroxisomal disorders, such as Zellweger syndrome [19]. EHHADH is essential for the production of medium-chain dicarboxylic acids [20]. Suto et al. [21] reported that the expression of *EHHADH* was decreased in human HCC, and *EHHADH* might be possible markers for the analysis of multistage hepatocarcinogenesis. Cytochrome P450 3A4 (CYP3A4), encoded by *CYP3A4*, is a member of the cytochrome P450 family of oxidizing enzymes. Cytochrome P450 enzymes metabolize approximately 60% of prescribed drugs, and CYP3A4 is responsible for about half of this metabolism [22]. CYP3A4 is involved in drug metabolism and synthesis of cholesterol, steroids and other lipids components. Many drugs are deactivated by CYP3A4, and there are also some drugs which are activated by the enzyme. Frequency and prognostic significant of CYP3A4-A-290G polymorphism were detected in acute myeloid leukemia [23]. The cytochrome P450 3A subfamily proteins are among the most important drug-metabolizing enzymes in human liver and are responsible for about half of all cytochrome P450-dependent drug oxidations [24]. In this study, we found the network of DEGs and DEMs were divided into 4 gene clusters, and *KNG1*, *CDK1*, *EHHADH* and *CYP3A4* were involved in more pairs in each cluster (Fig. 1), which suggested that they might play important roles in the occurrence of HCC. Furthermore, the expression of *CDK1* was significantly increased in HCC tissue, and expressions of *KNG1*, *EHHADH* and *CYP3A4* were significantly decreased (Table 7). Therefore, *KNG1*, *CDK1*, *EHHADH* and *CYP3A4* were potential biomarkers in HCC.

MiRNAs play roles in gene regulation by inhibiting protein translation of the targeting mRNAs, and aberrant

Table 6 The top 10 most significant enriched pathway of DEGs

Category	Term	Count	P
KEGG_PATHWAY	hsa01100:Metabolic pathways	142	2.62E-25
KEGG_PATHWAY	hsa00071:Fatty acid degradation	21	3.22E-15
KEGG_PATHWAY	hsa00982:Drug metabolism - cytochrome P450	25	5.27E-15
REACTOME_PATHWAY	R-HSA-211981:Xenobiotics	14	2.02E-13
KEGG_PATHWAY	hsa05204:Chemical carcinogenesis	25	3.29E-13
KEGG_PATHWAY	hsa00980:Metabolism of xenobiotics by cytochrome P450	24	4.49E-13
KEGG_PATHWAY	hsa00830:Retinol metabolism	22	2.06E-12
KEGG_PATHWAY	hsa00280:Valine,leucine and isoleucine degradation	17	3.86E-10
KEGG_PATHWAY	hsa01130:Biosynthesis of antibiotics	35	9.01E-10
REACTOME_PATHWAY	R-HSA-5661231:R-HSA-5661231	9	2.77E-09

DEGs, differentially expressed genes

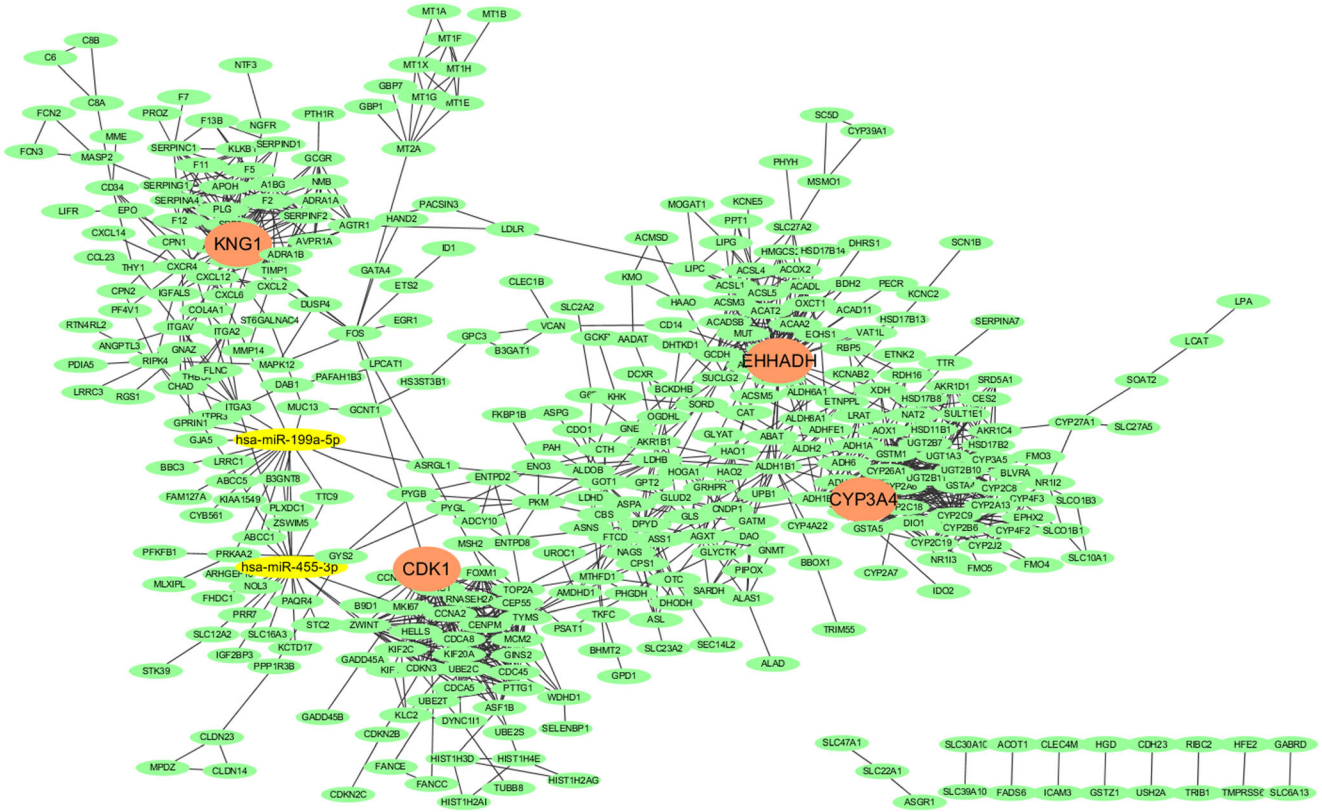

Fig. 1 The regulated network of DEGs and DEMs

expression has been implicated in a number of diseases including cancers [25]. Partially complementary microRNAs can speed up deadenylation, causing mRNAs to be degraded sooner [26]. Besides, miRNAs occasionally also cause histone modification and DNA methylation of promoter sites, which affects the expression of target genes [27]. Here, we identified the DEMs in HCC tissues compared with normal liver tissues, and predicted the targets of them. After constructed the regulated network of DEGs and DEMs, *hsa-miR-199a-5p* and *hsa-miR-455-3p* were found to be the only miRNAs and they can connect *KNG1* gene cluster and *CDK1* gene cluster. *Hsa-miR-199a-5p* could influence breast cancer cell invasiveness and might therefore be a potential drugable regulator of tumour progression and invasion [28]. *Hsa-miR-199a-5p* has been reported to be decreased in HCC compared to normal tissues, and transfection of miR-199a-5p inhibited invasion of HepG2 cells [29]. *Hsa-miR-455-3p* was reported to be overexpressed in punch biopsies of basal cell carcinoma and buccal mucosa of oral submucous fibrosis patients [30, 31]. Although no reports were retrieved to explore the relationship between *hsa-miR-455-3p* and HCC, we suspected that *hsa-miR-199a-5p* and *hsa-miR-455-3p* might be potential biomarkers in the occurrence of HCC based on our results.

In conclusion, our study suggested that *KNG1*, *CDK1*, *EHHADH*, *CYP3A4, hsa-miR-199a-5p* and *hsa-miR-455-3p* might be potential biomarkers in the diagnosis of HCC. Moreover, further functional researches and clinical supports were needed.

Table 7 The expression levels of *KNG1*, *CDK1*, *EHHADH*, *CYP3A4, hsa-miR-199a-5p* and *hsa-miR-455-3p*

Group	Relative expression values					
	KNG1	CDK1	EHHADH	CYP3A4	hsa-miR-199a-5p	hsa-miR-455-3p
HCC group	2.53 ± 0.32	2.64 ± 0.41	1.28 ± 0.17	2.31 ± 0.38	1.04 ± 0.11	1.93 ± 0.18
Control group	3.71 ± 0.95	0.38 ± 0.02	2.03 ± 0.31	4.56 ± 1.30	1.95 ± 0.31	2.66 ± 0.43
P	0.031	0.019	0.043	0.0026	0.0035	0.029
T	−2.82	3.22	−2.58	−5.04	−5.17	−3.00

Acknowledgements We would like to thank all the members of our research group for their enthusiastic participation in this study.

Funding Tianjin Clinical Research Center for Organ Transplantation (Grant No.15ZXLCSY00070); International Science and Technology Cooperation Program of China (Grant No. 2015DFG31850); National Key Clinical Specialty Construction Project of Organ Transplantation Department (Grant No.2013544); Science and Technology Fund of Tianjin Municipal Health Bureau (Grant No. 2013 KZ034); The study of precisely medical technology in the perioperative period of liver transplantation for liver cancer (Grant No. 17ZXMFSY00040); Spring Buds Project (Grant No. CL201801); General Program of The National Natural Science Foundation of China (Grant No. 81870444); Trial Registration Clinical Trial Registration URL: http://www.chictr.org.cn/showproj.aspx?proj=19662 webcite Trial Registration number: ChiCTR-ROR-17012157.

Data Availability The datasets used during the present study are available from the corresponding author upon reasonable request.

Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. McGuire S (2016) World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. Advances in Nutrition (Bethesda, Md) 7:418–419
2. Ferlay J, Soerjomataram I, Dikshit R et al (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer 136:E359–E386
3. Siegel RL, Miller KD, Jemal A (2017) Cancer statistics, 2017. CA Cancer J Clin 67(9):7–30
4. Diaz-Gonzalez A, Forner A, Rodriguez de Lope C, Varela M (2016) New challenges in clinical research on hepatocellular carcinoma. Revista Espanola De Enfermedades Digestivas : Organo Oficial De La Sociedad Espanola De Patologia Digestiva 108:485–493
5. Li D, Satomura S (2015) Biomarkers for hepatocellular carcinoma (HCC): an update. Adv Exp Med Biol 867:179–193
6. Kiyokawa H, Yasuda H (2017) Serum Monomeric Laminin-Gamma2 as a Novel Biomarker for Hepatocellular Carcinoma. Cancer Science 108:1432–1439
7. Shaker O, Alhelf M, Morcos G, Elsharkawy A (2017) miRNA-101-1 and miRNA-221 expressions and their polymorphisms as biomarkers for early diagnosis of hepatocellular carcinoma. Infection, Genetics and Evolution : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases 51:173–181
8. Shen Q, Fan J, Yang XR et al (2012) Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. Lancet Oncol 13:817–826
9. Huang S, Jiang F, Wang Y et al (2017) Diagnostic performance of tumor markers AFP and PIVKA-II in Chinese hepatocellular carcinoma patients. Tumour Biology : the Journal of the International Society for Oncodevelopmental Biology and Medicine 39:1010428317705763
10. Saitta C, Raffa G, Alibrandi A et al (2017) PIVKA-II is a useful tool for diagnostic characterization of ultrasound-detected liver nodules in cirrhotic patients. Medicine 96:e7266
11. Iarovaia GA (2001) Kallikrein-kinin system: novel facts and concepts (literature review). Vopr Med Khim 47:20–42
12. Colman RW (2006) Regulation of angiogenesis by the kallikrein-kinin system. Curr Pharm Des 12:2599–2607
13. Quesada-Calvo F, Massot C, Bertrand V et al (2017) OLFM4, KNG1 and Sec24C identified by proteomics and immunohistochemistry as potential markers of early colorectal cancer stages. Clin Proteomics 14:9
14. Ren Y, Ji N, Kang X et al (2016) Aberrant ceRNA-mediated regulation of KNG1 contributes to glioblastoma-induced angiogenesis. Oncotarget
15. Cho SJ, Lee SS, Kim YJ et al (2010) Xylocydine, a novel Cdk inhibitor, is an effective inducer of apoptosis in hepatocellular carcinoma cells in vitro and in vivo. Cancer Lett 287:196–206
16. Luo HB, Chen GW, Shao YX, Li Z, Liu M, Liu PQ (2012) 3D-QSAR studies on pyrimidine analogues as cyclin-dependent kinase 1 inhibitors. Shenzhen Daxue Xuebao 29:438–443
17. Zhao J, Han SX, Ma JL et al (2013) The role of CDK1 in apoptin-induced apoptosis in hepatocellular carcinoma cells. Oncol Rep 30:253–259
18. Yi Z, Wei H, Yan R et al (2015) miR-582-5p inhibits proliferation of hepatocellular carcinoma by targeting CDK1 and AKT3. Tumor Biol 36:8309–8316
19. Baes M, Van Veldhoven PP (2016) Hepatic dysfunction in peroxisomal disorders. BBA - Molecular Cell Research 1863:956–970
20. Houten SM, Denis S, Argmann CA (2012) Peroxisomal L-bifunctional enzyme (Ehhadh) is essential for the production of medium-chain dicarboxylic acids. J Lipid Res 53:1296–1303
21. Suto K, Kajihara-Kano H, Yokoyama Y et al (1999) Decreased expression of the peroxisomal bifunctional enzyme and carbonyl reductase in human hepatocellular carcinomas. J Cancer Res Clin Oncol 125:83–88
22. Zanger UM, Schwab M (2013) Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 138:103–141
23. Ali GT, Al-Azhary NM, Mokhtar DA (2014) Frequency and prognostic significant of CYP3A4-a-290G polymorphism in acute myeloid leukemia. J Adv Res 5:657–661
24. Rodrigues RM, De Kock J, Doktorova TY, Rogiers V, Vanhaecke T (2015) Measurement of cytochrome P450 enzyme induction and inhibition in human hepatoma cells. Methods Mol Biol 1250:279–285
25. Williams AE (2008) Functional aspects of animal microRNAs. Cell Mol Life Sci 65:545–562
26. Eulalio A, Huntzinger E, Nishihara T, Rehwinkel J, Fauser M, Izaurralde E (2009) Deadenylation is a widespread effect of miRNA regulation. RNA (New York, NY) 15:21–32
27. Hawkins PG, Morris KV (2008) RNA and transcriptional modulation of gene expression. Cell cycle (Georgetown, Tex) 7:602–607
28. Schultz S, Neubauer H, Bartsch H et al (2014) Hsa-miR-199a-5p is functionally relevant in progression from ductal carcinoma in situ to invasive breast tumours. Oncology Research & Treatment 37:68–68
29. Shen Q, Cicinnati VR, Zhang X et al (2010) Role of microRNA-199a-5p and discoidin domain receptor 1 in human hepatocellular carcinoma invasion. Mol Cancer 9:227
30. Sand M, Skrygan M, Sand D et al (2012) Expression of microRNAs in basal cell carcinoma. Br J Dermatol 167:847–855
31. Chickooree D, Zhu K, Ram V, Wu HJ, He ZJ, Zhang S (2016) A preliminary microarray assay of the miRNA expression signatures in buccal mucosa of oral submucous fibrosis patients. J Oral Pathol Med 45:691–697