

# Comprehensive analysis of key genes, microRNAs and long non-coding RNAs in hepatocellular carcinoma

Baoqi Shi, Xuejun Zhang, Lumeng Chao, Yu Zheng, Yongsheng Tan, Liang Wang and Wei Zhang

Department of Intervention, Inner Mongolia People's Hospital, Hohhot, China

## Keywords

hepatocellular carcinoma; long non-coding RNA; miRNA; mRNA; TCGA

## Correspondence

X. Zhang, Department of Intervention, Inner Mongolia People's Hospital, No. 20, Zhao Wuda Road, Saihan District, Hohhot 010010, China  
Tel: +86 13804745122  
E-mails: zhangxuejun\_doctor@163.com or zhangxj5122@126.com

(Received 23 February 2018, revised 10 June 2018, accepted 25 June 2018)

doi:10.1002/2211-5463.12483

Human hepatocellular carcinoma (HCC) is a common aggressive cancer whose molecular mechanism remains elusive. We aimed to identify the key genes, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) involved with HCC. We obtained mRNA, miRNA and lncRNA profiles for HCC from The Cancer Genome Atlas and then identified differentially expressed mRNAs (DEmRNAs), miRNAs (DEmiRNAs) and lncRNAs (DElncRNAs). We performed functional annotation of DEmRNAs and then constructed HCC-specific DEmiRNA–DEmRNA, DEmiRNA–DElncRNA and DElncRNA–DEmiRNA–DEmRNA interaction networks. We searched for nearby target *cis*-DEmRNAs of DElncRNAs and performed receiver operating characteristic and survival analyses. A total of 1239 DEmRNAs, 33 DEmiRNAs and 167 DElncRNAs in HCC were obtained. Retinol metabolism [false discovery rate (FDR) =  $7.02 \times 10^{-14}$ ] and metabolism of xenobiotics by cytochrome P450 (FDR =  $7.30 \times 10^{-11}$ ) were two significantly enriched pathways in HCC. We obtained 545 DEmiRNA–DEmRNA pairs that consisted of 258 DEmRNAs and 28 DEmiRNAs in HCC. miR-424, miR-93 and miR-3607 are three hub DEmiRNAs of the HCC-specific DEmiRNA–DEmRNA interaction network. HAND2-AS1/ENSG00000232855–miR-93–*LRAT/RND3*, ENSG00000232855–miR-877–*RCAN1* and ENSG00000232855–miR-224–*RND3* interactions were found in the HCC-specific DElncRNA–DEmiRNA–DEmRNA interaction network. A total of three DElncRNA–nearby target DEmRNA pairs (*HCG25–KIFC1*, *LOC105378687–CDC20* and *LOC101927043–EPCAM*) in HCC were obtained. Diagnostic and prognostic values of several selected DElncRNAs, DEmRNAs and DEmiRNAs for HCC were assessed. Our study identified several DEmRNAs, DEmiRNAs and DElncRNAs with great diagnostic or prognostic value for HCC, which may facilitate studies into the molecular mechanisms, and development of potential biomarkers and therapeutic target sites for HCC.

Human hepatocellular carcinoma (HCC) is the fifth most common cancer as well as the third leading cause of cancer-related mortality worldwide [1]. It is a highly

aggressive cancer that is characterized by fast infiltrating growth, early metastasis, high-grade malignancy and poor prognosis [2]. Only around 10–20% of

## Abbreviations

AUC, area under the ROC curve; CDC20, cell division cycle protein 20; DElncRNA, differentially expressed lncRNA; DEmiRNA, differentially expressed miRNA; DEmRNA, differentially expressed mRNA; EEC, endometrioid endometrial carcinoma; EPCAM, epithelial cell adhesion molecule; FC, fold change; FDR, false discovery rate; GO, Gene Ontology; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; KEGG, Kyoto Encyclopedia of Genes and Genomes; KIFC1, kinesin family member C1; lncRNA, long non-coding RNA; LRAT, lecithin retinol acyltransferase; miRNA, microRNA; RCAN1, regulator of calcineurin 1; RND3, Rho family GTPase 3; TCGA, The Cancer Genome Atlas.

patients with HCC are diagnosed at the early stage due to lack of effective diagnostic approaches [3,4]. Moreover, the long-term overall survival rate remains rather low despite various therapeutic strategies for HCC having been developed [5]. Hence, it is crucial to elucidate the mechanism and develop accurate diagnostic biomarkers and effective therapeutic strategies for HCC.

Previous studies have identified risk factors of HCC such as chronic infection with hepatitis B virus and hepatitis C virus, hepatocirrhosis induced by alcohol, other chronic inflammatory-related factors and hepatic regenerative changes [6–8]. However, the molecular mechanism of HCC remains largely unknown. Aberrantly expressed genes such as *RND3*, *LRAT*, *ECHS1*, *ACAA1*, *MT2A* and *MYC* have been demonstrated to be associated with the pathogenesis of HCC [9–11]. In addition, accumulated evidence has demonstrated that aberrantly expressed microRNAs (miRNAs), such as miR-21, miR-93, miR-424, miR-181b, miR-221, miR-222 and miR-122, were associated with the development and progression of HCC [12–14]. Long non-coding RNAs (lncRNAs) are a class of conserved non-protein-coding RNAs with more than 200 nucleotides that are broadly distributed in the human genome [15]. They involve many biological processes and could regulate gene expression in *cis* or in *trans* by diverse mechanisms [16]. They were reported to play key roles in various cancers such as colorectal cancer, breast cancer and HCC [17–19]. However, only a handful of HCC-associated lncRNAs, such as HULC, HOTAIR, MEG3, MVIH and MTIDP, have been investigated [17,18]. To better understand the mechanism of HCC, it is crucial to identify key genes, miRNAs and lncRNAs in HCC. Moreover, many previous studies focused on revealing the functions of each individual gene, miRNA and lncRNA in the process of HCC, and hence mechanistic relationships among them remain largely unknown.

In this study, comprehensive analysis of mRNA, miRNA and lncRNA profiling data of HCC from The Cancer Genome Atlas (TCGA) was performed. We identified differentially expressed mRNAs (DEmRNAs), miRNAs (DEmiRNAs) and lncRNAs (DElncRNAs) in HCC. Based on bioinformatics analysis, interactions among DEmRNAs, DEmiRNAs and DElncRNAs were analyzed. Receiver operating characteristic (ROC) and survival analyses were performed to access the diagnostic and prognostic value of selected DElncRNAs, DEmRNAs and DEmiRNAs for HCC. Our study may provide new clues for exploring molecular mechanisms of HCC and developing HCC-associated diagnostic and therapeutic approaches.

## Materials and methods

### mRNA, miRNA and lncRNA profiles of HCC in TCGA

The Cancer Genome Atlas is a central bank for multidimensional data of various cancers at DNA, RNA and protein levels. In this study, the clinical information of patients with HCC was downloaded from TCGA data portal (<http://tcga-data.nci.nih.gov/>). RSEM-normalized mRNA and lncRNA expression profiles (Level 3-IlluminaHiSeq\_RNA-SeqV2 data) and miRNA expression profile (Level 3-IlluminaHiSeq-miRNASeq data) between HCC and adjacent normal tissues were downloaded from TCGA data portal (<http://tcga-data.nci.nih.gov/>) as well.

### DEmRNAs, DEmiRNAs and DElncRNAs in HCC compared with adjacent tissues

Before identifying the DEmRNAs, DEmiRNAs and DElncRNAs between HCC and normal tissues, we firstly filtered the difficultly detected miRNAs, mRNAs and lncRNAs (miRNAs, mRNAs and lncRNAs with read count value = 0 in more than 10% of HCC cases or in more than 10% of normal tissues).

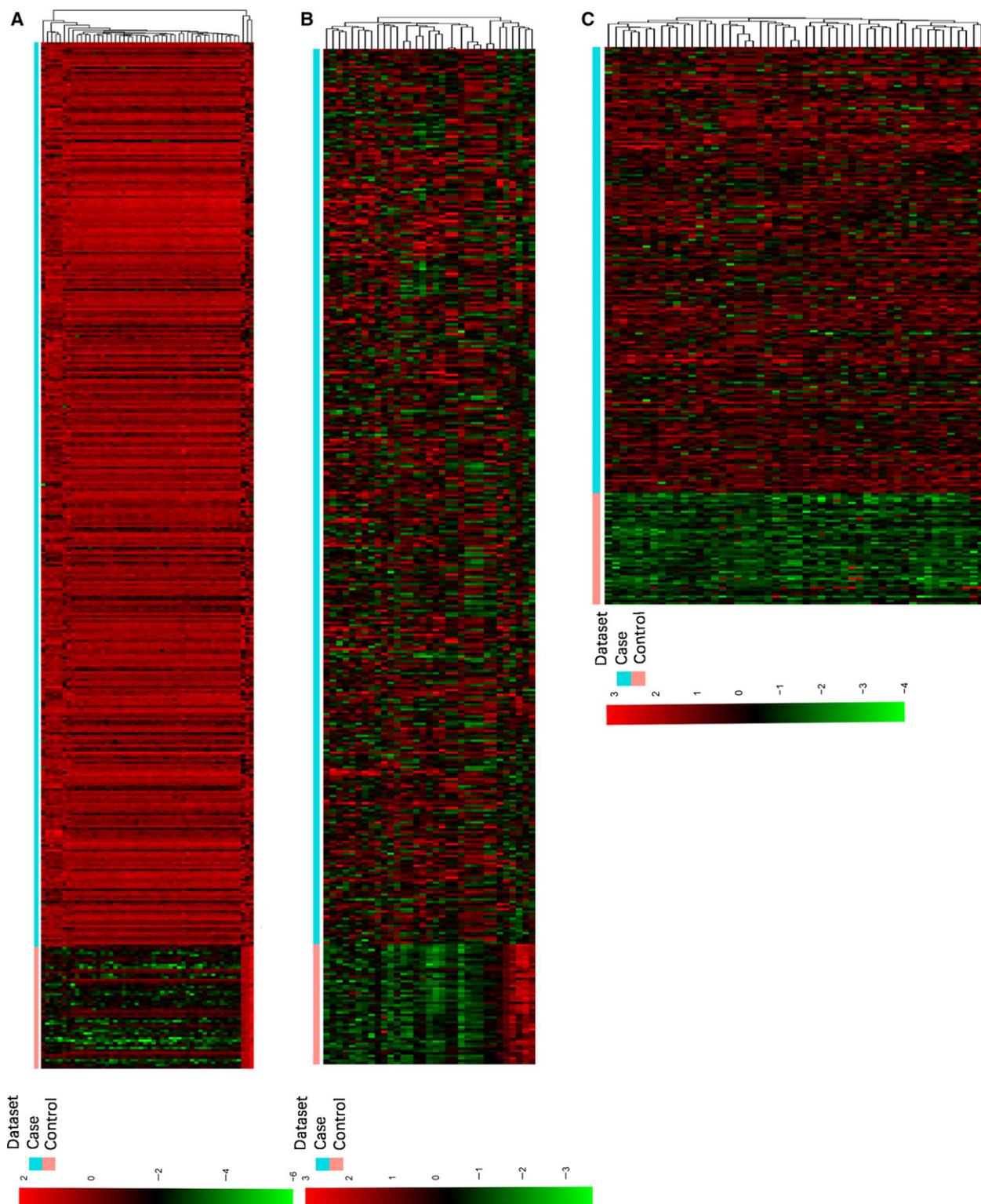
Then, based on the read count of each sample, the DEmRNAs and DEmiRNAs in HCC compared with adjacent tissues were calculated with the R-bioconductor package DESEQ2 [20] with false discovery rate (FDR) < 0.01 and abs [log<sub>2</sub> fold change (FC)] > 1.5. Based on the BAM files, we used reads per kilobase per million reads (RPKM) to quantify the expression levels of lncRNAs. Student's *t* test was performed to obtain *P* values. Using the Benjamini and Hochberg method, multiple comparisons were performed to obtain the FDR [21]. The threshold for the DElncRNAs was FDR < 0.01 and abs (log<sub>2</sub> FC) > 1.5 as well.

### Functional annotation of DEmRNAs between HCC and normal tissues

Functional annotation, including Gene Ontology (GO) classification and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEmRNAs between HCC and normal tissues, was conducted using online software GENECODIS (<http://genecodis.cnb.csic.es/analysis/>). Statistical significance was defined as FDR < 0.05.

### HCC-specific DEmiRNA–DEmRNA interaction network

Firstly, pairwise Pearson correlation coefficients between DEmRNAs and DEmiRNAs were calculated. DEmiRNA–DEmRNA pairs with *P* < 0.05 and *r* < 0 were served as significant negative DEmiRNA–DEmRNA co-expression



**Fig. 1.** Hierarchical clustering analysis of the DEmRNAs, DE miRNAs and DE lncRNAs in HCC and adjacent normal tissues. Rows and columns represent samples and DEmRNAs, DE miRNAs, and DE lncRNAs, respectively. Red and green represent up- and downregulation, respectively. Case: HCC tissues; control: adjacent normal tissues. (A) Hierarchical clustering analysis of the top 50 DEmRNAs in HCC; (B) hierarchical clustering analysis of the DE miRNAs in HCC; (C) hierarchical clustering analysis of the top 50 DE lncRNAs in HCC.

pairs. Then, the putative targeted DEmRNAs of DEmiRNAs were predicted by six bioinformatic algorithms (RNA22, MIRANDA, MIRDDB, MIRWALK, PICTAR2 and TARGETSCAN) of MIRWALK2.0 (<http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/mir-mir-self.html>). Targets recorded by  $\geq 4$  algorithms were served as target DEmRNAs of DEmiRNAs. The confirmed target DEmRNAs of DEmiRNAs were obtained by MIRWALK2.0 as well. Finally, DEmiRNA–DEmRNA co-expression pairs were obtained whose DEmRNA was not only negatively co-expressed with DEmiRNAs but also the predicted targets of this DEmiRNA with  $\geq 4$  algorithms or confirmed targets of this DEmiRNA. Based on these DEmiRNA–DEmRNA pairs, the DEmiRNA–DEmRNA interaction network was constructed and visualized using CYTOSCAPE software (<http://www.cytoscape.org/>).

### HCC-specific DElncRNA–DEmiRNA interaction network

Firstly, pairwise Pearson correlation coefficients between DElncRNAs and DEmiRNAs were calculated. DElncRNA–DEmiRNA pairs with  $P < 0.05$  and  $r < 0$  were served as significant negative DElncRNA–DEmiRNA co-expression pairs. Then, the putative targeted DElncRNAs of DEmiRNAs were predicted by miRWalk of MIRWALK2.0. Finally, DElncRNA–DEmiRNA pairs whose DElncRNA was not only negatively co-expressed with DEmiRNAs but also the predicted targets of this DEmiRNA by miRWalk were obtained. Based on these DElncRNA–DEmiRNA

pairs, the DElncRNA–DEmiRNA interaction network was constructed and visualized using CYTOSCAPE software.

### HCC-specific DElncRNA–DEmiRNA–DEmRNA interaction network

The HCC-specific DElncRNA–DEmiRNA–DEmRNA interaction network was constructed by merging the HCC-specific DEmiRNA–DEmRNA interaction network and DElncRNA–DEmiRNA interaction network based on the common DEmiRNAs.

### Nearby targeted DEmRNAs of DElncRNAs in HCC

To identify the target DEmRNAs of DElncRNAs by *cis*-regulatory effects, we searched the DEmRNAs transcribed within a 200-kb window up- or downstream of DElncRNAs that were served as nearby *cis*-targeted DEmRNAs of DElncRNAs.

### ROC analysis

In order to access the diagnostic value of DElncRNAs, DEmRNAs and DEmiRNAs for HCC, respectively, the PROC package was used to calculate ROC, and the area under the ROC curve (AUC) was further calculated. When AUC value was greater than 0.8, the DElncRNAs/DEmRNAs/DEmiRNAs were considered capable of distinguishing patients with HCC and normal controls with excellent specificity and sensitivity.

**Table 1.** Top 10 up- and downregulated DEmRNAs between HCC and normal tissues.

Gene ID	Symbol	Log FC	<i>P</i> value	FDR	Regulation
1033	<i>CDKN3</i>	4.40	1.02E-106	1.50E-102	Up
83540	<i>NUF2</i>	4.77	9.25E-102	6.76E-98	Up
1306	<i>COL15A1</i>	4.28	2.30E-100	1.12E-96	Up
83483	<i>PLVAP</i>	2.89	1.82E-98	6.66E-95	Up
24137	<i>KIF4A</i>	4.35	7.46E-96	2.18E-92	Up
29089	<i>UBE2T</i>	3.43	1.02E-94	2.47E-91	Up
1063	<i>CENPF</i>	3.94	3.91E-93	8.17E-90	Up
3833	<i>KIFC1</i>	3.96	5.94E-93	1.08E-89	Up
9833	<i>MELK</i>	4.17	2.48E-90	4.02E-87	Up
11004	<i>KIF2C</i>	4.38	5.64E-90	8.24E-87	Up
11093	<i>ADAMTS13</i>	−2.90	1.25E-77	4.95E-75	Down
170392	<i>OIT3</i>	−3.56	2.08E-73	6.19E-71	Down
64651	<i>CSRNP1</i>	−2.24	6.54E-60	1.02E-57	Down
1893	<i>ECM1</i>	−3.02	2.44E-54	3.05E-52	Down
1827	<i>RCAN1</i>	−2.39	9.08E-53	1.05E-50	Down
5199	<i>CFP</i>	−3.33	6.69E-52	7.40E-50	Down
390	<i>RND3</i>	−2.47	2.22E-51	2.35E-49	Down
83854	<i>ANGPTL6</i>	−3.02	7.92E-49	7.51E-47	Down
7538	<i>ZFP36</i>	−2.15	6.23E-47	5.42E-45	Down
9227	<i>LRAT</i>	−3.22	2.35E-45	1.82E-43	Down

## Survival analysis

Using survival (<https://cran.r-project.org/web/packages/survival/index.html>) in R, the prognostic value of selected DElncRNAs, DEmRNAs and DEmiRNAs for patients with HCC was analyzed.

## Results

### DEmRNAs, DEmiRNAs and DElncRNAs in HCC

Data for a total of 377 patients with HCC were downloaded from TCGA data portal. From these there were obtained the mRNA expression profile of HCC tissues of 371 patients with HCC and 50 adjacent tissues, the miRNA expression profile of HCC tissues of 372 patients with HCC and 50 adjacent tissues and the lncRNA expression profile of HCC tissues of 200 patients with HCC and 50 adjacent tissues.

After filtering the difficultly detected miRNAs, mRNAs and lncRNAs, a total of 311 miRNAs, 14 607 mRNAs and 2152 lncRNAs were retained for analysis. A total of 1239 DEmRNAs (865 of them upregulated and 374 of them downregulated), 33 DEmiRNAs (29 upregulated and four downregulated) and 167 DElncRNAs (165 upregulated and two downregulated) in HCC were obtained. A heat-map of DEmRNAs, DEmiRNAs and DElncRNAs in HCC is displayed in Fig. 1. The top 10 up- and downregulated DEmRNAs and DEmiRNAs, and the top 20 DElncRNAs between HCC and normal tissues are displayed in Tables 1–3, respectively.

### Functional annotation of DEmRNAs between HCC and normal tissues

Functional annotation of DEmRNAs between HCC and normal tissues indicated that mitotic cell cycle (FDR =  $4.56 \times 10^{-36}$ ), protein binding (FDR =  $2.16 \times 10^{-26}$ ), and cytoplasm (FDR =  $1.25 \times 10^{-34}$ ) were significantly enriched GO terms (Fig. 2A–C). Retinol metabolism (FDR =  $7.02 \times 10^{-14}$ ) and metabolism of xenobiotics by cytochrome P450 (FDR =  $7.30 \times 10^{-11}$ ) were two significantly enriched pathways (Fig. 2D,E).

### HCC-specific DEmiRNA–DEmRNA interaction network

Firstly, we obtained 7996 negative DEmiRNA–DEmRNA co-expression pairs with  $P < 0.05$  and  $r < 0$ . Then, a total of 1142 DEmiRNA-target DEmRNA

pairs with predicted  $\geq 4$  algorithms were obtained. Finally, 545 DEmiRNA–DEmRNA pairs were obtained whose DEmRNA was not only negatively co-expressed with DEmiRNAs but also the predicted targets of this DEmiRNA with  $\geq 4$  algorithms. These 545 DEmiRNA–DEmRNA pairs consisted of 258 DEmRNAs (88 upregulated and 170 downregulated) and 28 DEmiRNAs (25 upregulated and three downregulated) in HCC. The HCC-specific DEmiRNA–DEmRNA interaction network is displayed in Fig. 3. mir-424 (degree = 56), miR-93 (degree = 51), and miR-3607 (degree = 37) are three hub DEmiRNAs.

### HCC-specific DElncRNA–DEmiRNA interaction network

Firstly, we obtained 1258 negative DElncRNA–DEmiRNA co-expression pairs with  $P < 0.05$  and  $r < 0$ . Then, a total of 7090 DEmiRNA-target

**Table 2.** DEmiRNAs between HCC and normal tissues.

DEmiRNA	Log FC	<i>P</i> value	FDR	Regulation
hsa-mir-424	−2.21	5.92E-61	1.84E-58	Down
hsa-mir-10b	3.59	6.75E-59	1.05E-56	Up
hsa-mir-21	1.84	6.65E-55	6.89E-53	Up
hsa-mir-93	1.71	2.22E-51	1.73E-49	Up
hsa-mir-589	1.58	1.89E-50	1.18E-48	Up
hsa-mir-224	3.26	6.52E-47	3.38E-45	Up
hsa-mir-183	3.86	2.29E-46	1.02E-44	Up
hsa-mir-1269	5.56	8.76E-46	3.41E-44	Up
hsa-mir-96	3.75	3.42E-40	1.18E-38	Up
hsa-mir-500a	1.60	4.26E-40	1.32E-38	Up
hsa-mir-182	3.37	5.13E-39	1.45E-37	Up
hsa-mir-452	2.50	2.09E-36	5.40E-35	Up
hsa-mir-221	1.57	5.83E-30	1.13E-28	Up
hsa-mir-217	4.00	1.11E-27	1.93E-26	Up
hsa-mir-1180	1.77	8.59E-27	1.34E-25	Up
hsa-mir-9-1	3.23	1.31E-26	1.85E-25	Up
hsa-mir-9-2	3.22	1.49E-26	2.02E-25	Up
hsa-mir-196b	3.28	5.01E-26	6.00E-25	Up
hsa-mir-1266	2.06	6.79E-26	7.54E-25	Up
hsa-mir-3200	2.54	3.87E-23	3.89E-22	Up
hsa-mir-877	1.80	1.04E-21	9.26E-21	Up
hsa-mir-3677	1.65	5.26E-21	4.42E-20	Up
hsa-mir-18a	1.67	7.16E-20	5.57E-19	Up
hsa-mir-216a	3.45	1.48E-19	1.10E-18	Up
hsa-mir-19a	1.55	2.90E-19	2.05E-18	Up
hsa-mir-3607	−1.65	1.06E-16	6.12E-16	Down
hsa-mir-1274b	−1.52	1.83E-15	9.81E-15	Down
hsa-mir-508	2.19	6.42E-15	3.33E-14	Up
hsa-mir-937	1.68	1.39E-13	6.17E-13	Up
hsa-mir-1226	1.72	1.87E-13	7.98E-13	Up
hsa-mir-3648	−1.52	2.81E-11	1.03E-10	Down
hsa-mir-431	1.53	3.05E-08	8.54E-08	Up
hsa-mir-483	1.79	2.02E-05	4.29E-05	Up

DElncRNA pairs were obtained by MIRWALK. Finally, we obtained 342 DEmiRNA–DElncRNA pairs whose DElncRNA was not only negatively coexpressed with DEmiRNA but also the predicted targets of this DEmiRNA based on MIRWALK. The HCC-specific DElncRNA–DEmiRNA interaction network consisted of 260 nodes and 342 edges (Fig. 4). miR-424 (degree = 171) and miR-3648 (degree = 11) were hub DEmiRNAs of an HCC-specific DElncRNA–DEmiRNA interaction network.

### HCC-specific DElncRNA–DEmiRNA–DEmRNA interaction network

The HCC-specific DElncRNA–DEmiRNA–DEmRNA interaction network consisted of 417 nodes and 651 edges. HAND2-AS1/ENSG00000232855–miR-93–lecithin retinol acyltransferase (LRAT)/Rho family GTPase 3 (RND3), ENSG00000232855–miR-877–regulator of calcineurin 1 (RCAN1) and ENSG00000232855–miR-224–RND3 interactions were found in this HCC-specific DElncRNA–DEmiRNA–DEmRNA interaction network (Fig. 5).

### Nearby targeted DEmRNAs of DElncRNAs in HCC

A total of three DEmRNAs transcribed within a 200-kb window up- or downstream of three DElncRNAs in HCC were obtained. HCG25–kinesin family member C1 (KIFC1), LOC105378687–cell division cycle protein

20 (CDC20) and LOC101927043–epithelial cell adhesion molecule (EPCAM) are three DElncRNA–nearby target DEmRNA pairs (Table 4).

### ROC analysis

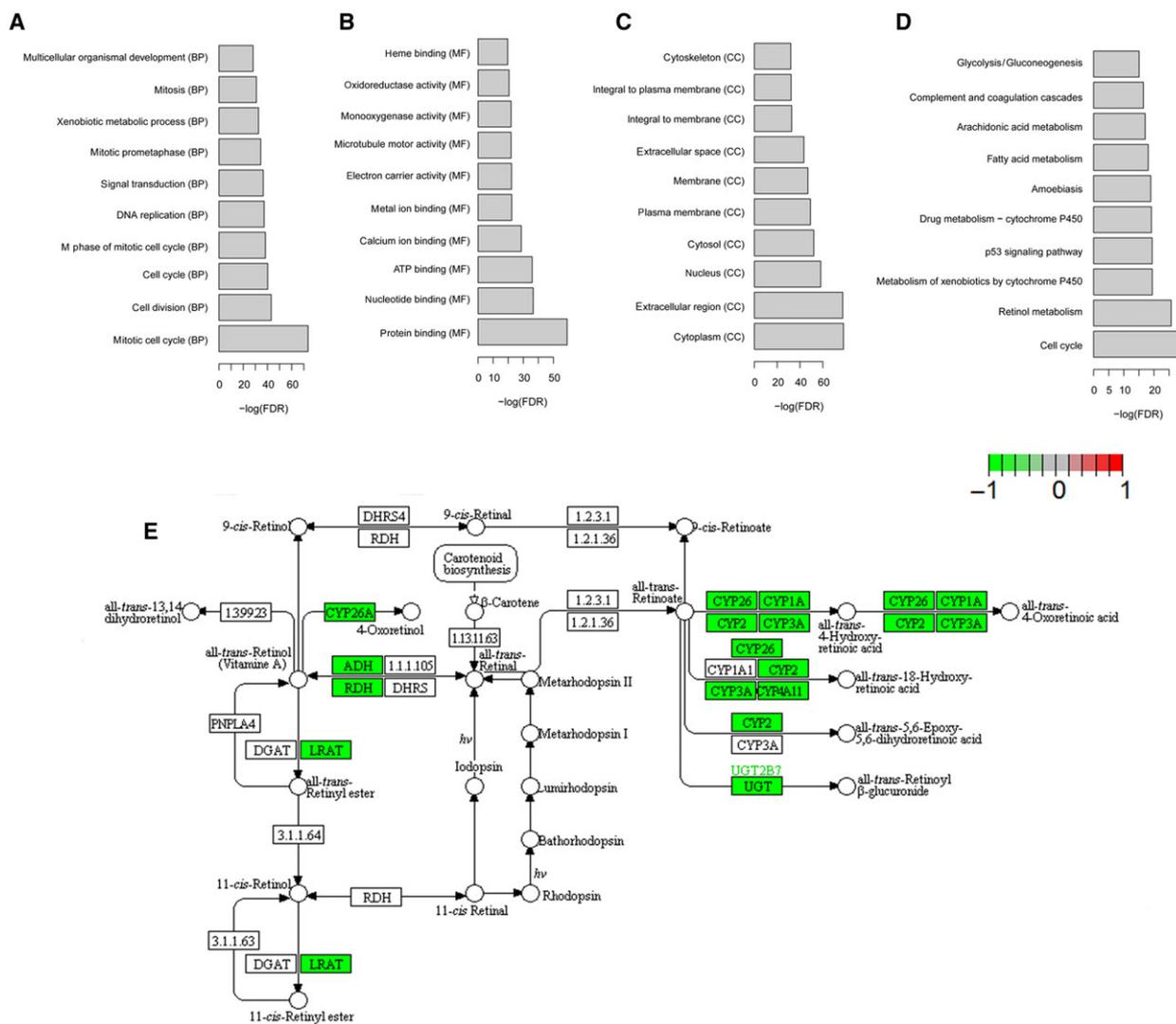
ROC curve analysis was performed to evaluate the diagnostic value of five DElncRNAs (HAND2-AS1, ENSG00000232855, HCG25, LOC105378687 and LOC101927043), five DEmRNAs (RND3, LART, RCAN1, KIFC1 and CDC20) and four DEmiRNAs (miR-424, miR-93, miR-224 and miR-877) for HCC. Except for LOC101927043 and miR-877, the other four DElncRNAs (HAND2-AS1, ENSG00000232855, HCG25 and LOC105378687), five DEmRNAs (RND3, LART, RCAN1, KIFC1 and CDC20) and three DEmiRNAs (miR-424, miR-93 and miR-224) have great diagnostic value for HCC with AUC more than 0.8 (Fig. 6).

### Survival analysis

Survival analysis was performed to evaluate the prognostic value of five DElncRNAs (HAND2-AS1, ENSG00000232855, HCG25, LOC105378687 and LOC101927043), five DEmRNAs (RND3, LART, RCAN1, KIFC1 and CDC20) and four DEmiRNAs (miR-424, miR-93, miR-224 and miR-877) for HCC. Only two DEmRNAs (CDC20 and KIFC1) and miR-877 have prognostic value for HCC. High expression of CDC20 ( $P = 1.03 \times 10^{-6}$ ), KIFC1

**Table 3.** Top 20 DElncRNAs between HCC and normal tissues.

ENSG	ID	Symbol	Log FC	P value	FDR	Regulation
ENSG00000267080	339201	ASB16-AS1	1.52	2.51E-38	1.80E-35	Up
ENSG00000212694	338799	LINC01089	2.21	5.18E-36	1.59E-33	Up
ENSG00000206573	440944	THUMPDP3-AS1	1.67	1.00E-32	1.32E-30	Up
ENSG00000232995	8490	RGS5	1.99	1.05E-32	1.32E-30	Up
ENSG00000249592	100129917	LOC100129917	1.66	1.32E-32	1.58E-30	Up
ENSG00000234608	51275	MAPKAPK5-AS1	1.54	1.44E-32	1.63E-30	Up
ENSG00000228288	100506696	PCAT6	2.34	5.79E-31	4.29E-29	Up
ENSG00000228265	101926888	RALY-AS1	1.52	3.47E-30	2.07E-28	Up
ENSG00000213742	102724826	ZNF337-AS1	1.68	3.29E-30	2.07E-28	Up
ENSG00000224424	100506637	PRKAR2A-AS1	2.26	5.79E-30	3.20E-28	Up
ENSG00000172965	541471	MIR4435-2HG	2.53	6.70E-30	3.52E-28	Up
ENSG00000234912	654434	SNHG20	1.67	2.32E-28	9.43E-27	Up
ENSG00000233527	101927599	ZNF529-AS1	1.69	5.34E-28	1.89E-26	Up
ENSG00000228106	102724017	LOC102724017	1.56	1.50E-27	4.82E-26	Up
ENSG00000250988	100505616	SNHG21	1.78	2.56E-27	7.66E-26	Up
ENSG00000226696	104355426	LENG8-AS1	2.19	3.76E-27	1.11E-25	Up
ENSG00000186615	100129075	KTN1-AS1	1.79	1.13E-26	2.98E-25	Up
ENSG00000198468	642946	FLVCR1-AS1	2.24	1.18E-26	3.06E-25	Up
ENSG00000232940	414765	HCG25	2.54	1.40E-26	3.58E-25	Up
ENSG00000234432	100129484	LOC100129484	1.88	1.95E-26	4.60E-25	Up



**Fig. 2.** Functional annotation of DEmRNAs between HCC and normal tissues. (A–D) The significantly enriched biological process (A), molecular function (B), cellular component (C) and KEGG pathways (D) for DEmRNAs between HCC and normal tissues. The x-axis shows  $-\log$  FDR and the y-axis shows GO terms or KEGG pathways. (E) The pathway of retinol metabolism. The red and green rectangles represent the particles that are regulated by up- and downregulated DEmRNAs, respectively, between HCC and normal tissues.

( $P = 8.58 \times 10^{-7}$ ) and miR-877 ( $P = 0.0108$ ) was significantly associated with a lower survival rate in patients with HCC (Fig. 7).

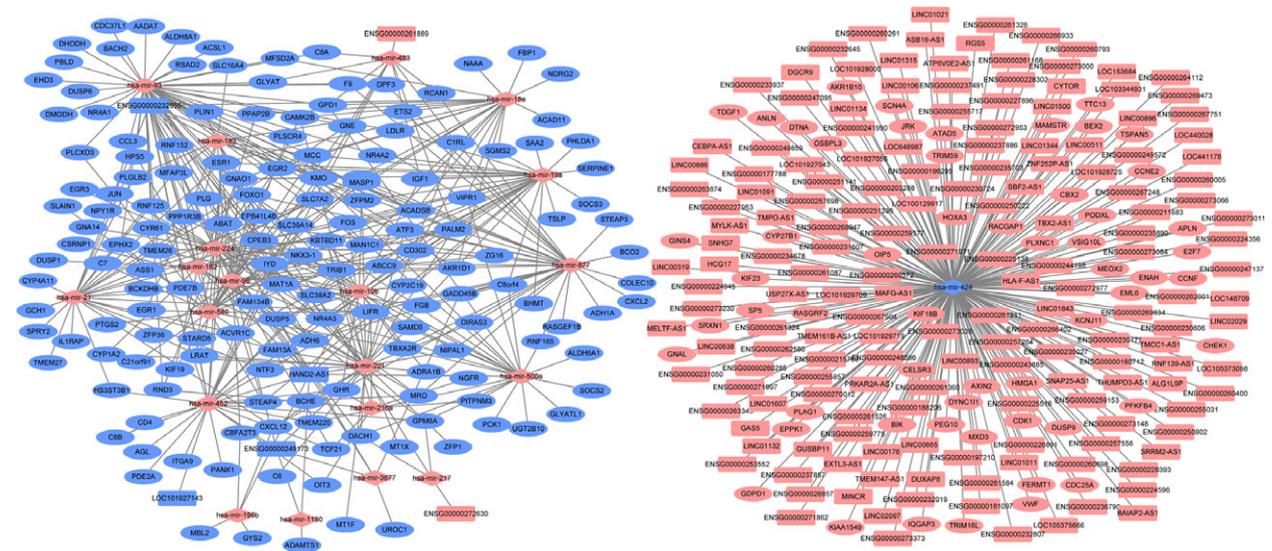
## Discussion

In this study, we identified DEmRNAs, DEMiRNAs and DELncRNAs between HCC and normal controls from TCGA. Their interactions and potential diagnostic and prognostic value for HCC were further examined by bioinformatics analysis.

Functional annotation of DEmRNAs indicated that retinol metabolism was a significantly enriched pathway in HCC. Retinoic acids have been demonstrated

to play an inhibitory role in carcinogenesis of various cancers, including HCC [22]. Inhibition of retinoic acid signaling in hepatocytes provoked the development of liver cancer in transgenic mice [23]. Metabolism of xenobiotics by cytochrome P450 was another significantly enriched pathway in HCC. This is a typical liver-function-specific pathway and has been indicated to play crucial roles in HCC [24]. The members of the cytochrome P450 (CYP) family have frequently been found to be involved in various biological processes that were found to be dysregulated in liver cancer [25]. Hence, DEmRNAs enriched in these two pathways might be regulators in HCC, and this needs further research.





**Fig. 5.** Hepatocellular carcinoma-specific DE miRNA–DE lncRNA–DE mRNA interaction network. Rectangles, rhombuses and ellipses represent DE lncRNAs, DE miRNAs and DE mRNAs, respectively. Red and blue represent up- and downregulation, respectively.

**Table 4.** DE lncRNA-nearby targeted DE mRNA pairs in HCC. Chr, chromosome.

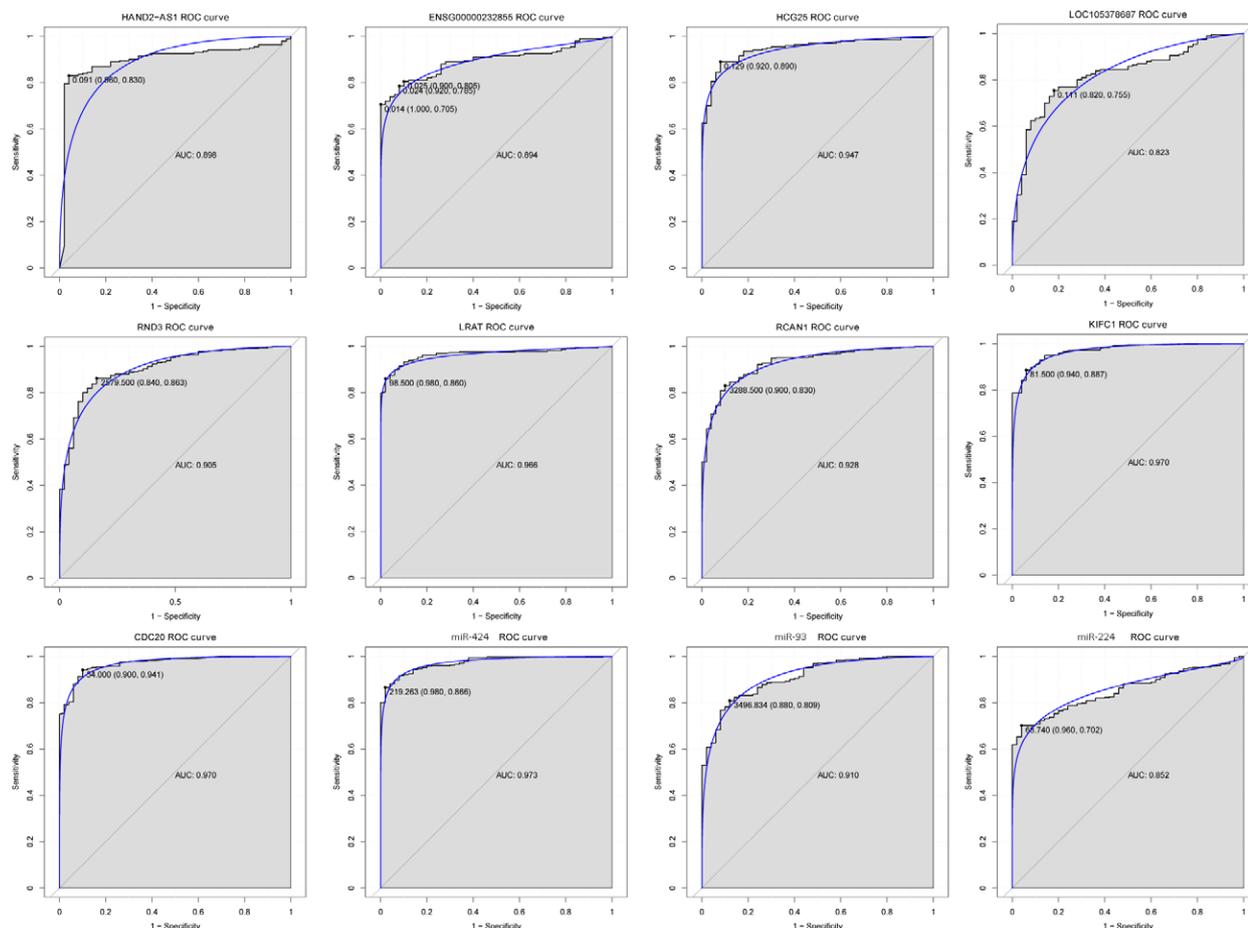
lncRNA					Nearby targeted mRNA		
Chr	lncRNA ENSG	lncRNA symbol	Start – 200 kb	End + 200 kb	mRNA symbol	Start	End
chr6	ENSG00000232940	HCG25	33049534	33454989	<i>KIFC1</i>	33391536	33409924
chr1	ENSG00000234694	LOC105378687	43154684	43558658	<i>CDC20</i>	43358955	43363203
chr2	ENSG00000234690	LOC101927043	46992405	47545074	<i>EPCAM</i>	47345158	47387601

suggesting their importance in HCC. Upregulated miR-93 has been found in patients with HCC in previous studies, which is consistent with the present study [14]. Increased miR-93 was associated with cell migration and invasion of HCC and serves as a potential marker of poor 5-year overall survival of patients with HCC [14,26]. Based on our DE miRNA–DE mRNA interaction network, miR-424, miR-93 and miR-224 had 56, 51 and 34 targeted DE mRNAs in HCC, respectively. *RND3* was a shared target of both miR-93 and miR-224. *LRAT* was another target of miR-93. Both *RND3* and *LRAT* are two downregulated DE mRNAs derived from the top 10 downregulated DE mRNAs and have great diagnostic value for HCC.

Previous studies have indicated that both *RND3* and *LRAT* are HCC-related genes. *RND3* is a member of the RND subfamily of the Rho GTPase family. *RND3* was significantly downregulated in HCC cell lines and tissues. HCC cell growth could be inhibited by knockdown of *RND3* [10]. *RND3* was speculated to regulate a switch to attenuate cell

growth and favor cell invasion and serve as a potential metastasis suppressor gene in HCC [10]. Retinoid is mainly stored in the liver in the form of retinyl ester in lipid droplets. Hepatic stellate cells (HSCs) serve as the major cells of retinoid storage within the liver [27]. Lack of retinoid-containing lipid droplets of HSCs has been observed in the development of liver disease leading to HCC [27]. As the sole enzyme that conducts the synthesis of hepatic retinyl ester, *LRAT* may play a key role in the pathogenesis of HCC [11]. Our study found that *LRAT* was downregulated in patients with HCC, which was consistent with a previous study [28]. Taken together, miR-93–*RND3*/*LRAT* and miR-224–*RND3* interactions may play crucial roles in HCC.

lncRNAs were reported to bind to miRNA and act as sponges for miRNAs [29]. By sharing common miRNA binding sites with mRNA targets, lncRNAs sequester and compete with miRNA to inhibit miRNA function and alleviate mRNA repression [30]. In the present study, we constructed the lncRNA–miRNA–



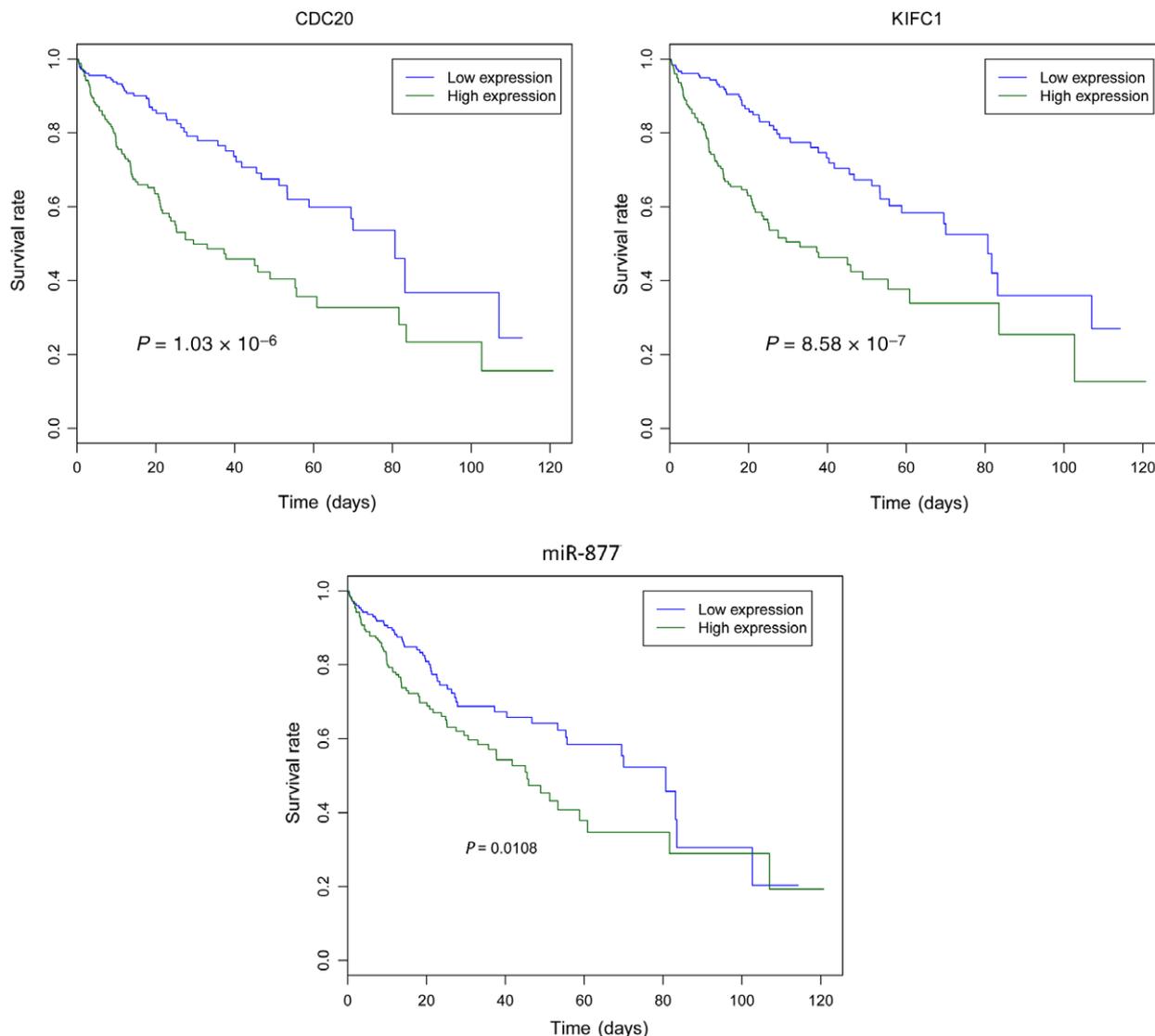
**Fig. 6.** ROC analysis of selected DE mRNAs, DE miRNAs and DE lncRNAs. ROC curves were used to show the diagnostic value of selected DE lncRNAs, DE mRNAs and DE miRNAs for HCC with sensitivity and specificity. The x-axis indicates 1 – specificity, and y-axis indicates sensitivity. Names of the DE lncRNAs, DE mRNAs and DE miRNAs are displayed above the ROC curve.

mRNA interaction network based on the shared common miRNAs.

Two downregulated lncRNAs (HAND2-AS1 and ENSG00000232855) with great diagnostic value for HCC were shared targets of both miR-93 and miR-244. HAND2-AS1 transcribed antisense adjacent to heart and neural crest derivatives expressed 2 (HAND2) in chromosome 4q33-34 [31]. HAND2-AS1 was reported to play an inhibiting role in migration and invasion of endometrioid endometrial carcinoma (EEC) cells by inactivating neuromedin U [31]. Down-regulated HAND2-AS1 has been found in EEC tissues [31]. Moreover, HAND2-AS1 was closely associated with tumor grade, lymph node metastasis and recurrence of EEC patients and serves as a potential prognostic biomarker [31]. A recent study indicated that HAND2-AS1 was also downregulated in HCC tissues, which was associated with migration of HCC cells [32]. In the present study, HAND2-AS1 was

downregulated in HCC, which provided evidence in support of the previous study. We speculate that HAND2-AS1 might be involved with the process of HCC by inhibiting miR-93 and miR-244 and competing with their targets such as LRAT and RND3. Like miR-93, ENSG00000232855 was speculated to play roles in HCC as well.

Additionally, ENSG00000232855 was a target of another HCC-related miRNA, miR-877. A previous study indicated that miR-877 plays a regulating role in cell proliferation, apoptosis and the cell cycle of HCC [33]. In this study, we highlighted the prognostic value of miR-877 for HCC. Considering targeted DE mRNAs of miR-877, RCAN1 was a downregulated DE mRNA derived from the top 10 downregulated DE mRNAs in HCC in the present study. Downregulation of RCAN1 has been found in HCC tissues. Based on the experiments *in vitro*, RCAN1 has an inhibitory role in cell proliferation, migration and invasion of



**Fig. 7.** Survival analysis of selected DEmRNAs, DE miRNAs, and DE lncRNAs. Survival curves were used to show the prognostic value of selected DEmRNAs and DE miRNAs for HCC. The x-axis indicated times (days), and y-axis indicated survival rate. Above the survival curves, names of DEmRNAs and DE miRNAs were displayed. High expression of CDC20 ( $P = 1.03 \times 10^{-6}$ ), KIFC1 ( $P = 8.58 \times 10^{-7}$ ), and miR-877 ( $P = 0.0108$ ) were significantly associated with lower survival rate in patients with HCC.

HCC cells [34]. ENSG00000232855–miR-877–*RCANI* interaction was speculated to play key roles in the process of HCC.

In addition, we obtained three DE lncRNA–nearby target DEmRNA pairs, namely HCG25–*KIFC1*, LOC105378687–*CDC20* and LOC101927043–*EPCAM*. *KIFC1* was widely overexpressed in various cancers such as breast cancer, non-small-cell lung cancer and gastric cancer, and was reported to be involved with the development and prognosis of cancers [35–37]. A recent study found that overexpressed *KIFC1* was found in HCC and was associated with shorter overall

survival time of patients with HCC [38]. Upregulated *KIFC1* was also found in HCC with both diagnostic and prognostic value for HCC in our study, which provided evidence in support of the previous study. There is no study report on the association between HCC and HCG25. *KIFC1* was a nearby target gene of HCG25 and HCG25 was significantly upregulated in HCC and has great diagnostic value for HCC. We speculate that HCG25 may regulate the process of HCC by its *cis*-regulatory role on the expression of *KIFC1*. As one of the key genes associated with the hepatocyte cell cycle, *CDC20* has been reported to be

involved with the development of HCC [39]. Silencing *CDC20* could delay hepatocellular mitotic progression and inhibit HCC cell proliferation [40,41]. In this study, both diagnostic and prognostic values of *CDC20* for HCC were observed. EPCAM is a cell surface glycoprotein that serves as a marker of cancer stem cells. Upregulated *EPCAM* has been found in HCC tissues compared with normal liver tissues. Moreover, *EPCAM* was associated with shorter survival of patients with HCC. We speculate that LOC105378687 and LOC101927043 may play roles in the development of HCC by interacting with *CDC20* and *EPCAM*, respectively.

## Conclusions

In conclusion, our study was a comprehensive analysis of key DEmRNAs, DE miRNAs and DE lncRNAs in HCC. Based on the bioinformatics analysis, several DEmRNAs, DE miRNAs and DE lncRNAs and their interactions may play important roles in the process of HCC, which has provided clues for exploring the molecular mechanisms of HCC. Moreover, diagnostic and prognostic values of several key DEmRNAs, DE miRNAs and DE lncRNAs for HCC were found in this study, which has made a contribution toward developing potential biomarkers and therapeutic target sites for HCC.

## Acknowledgements

We thank Medintell Bioinformatic Technology Co., Ltd, for assistance in data analysis.

## Author contributions

BS and XZ conceived and designed the project; XZ provided support for administration; BS, YZ and LC contributed reagents, materials and analysis tools; BS, YZ and LW collected the data; BS, YT and WZ analyzed and interpreted the data; all authors wrote and approved the final manuscript.

## References

- 1 Tan G, Wu L, Tan J, Zhang B, Tai WC, Xiong S, Chen W, Yang J and Li H (2016) MiR-1180 promotes apoptotic resistance to human hepatocellular carcinoma via activation of NF- $\kappa$ B signaling pathway. *Sci Rep* **6**, 22328.
- 2 Tanaka S and Arai S (2011) Molecular targeted therapy for hepatocellular carcinoma in the current and potential next strategies. *J Gastroenterol* **46**, 289–296.
- 3 Schwabe RF and Wang TC (2011) Targeting liver cancer: first steps toward a miRacle? *Cancer Cell* **20**, 698–699.
- 4 Wang PR, Xu M, Toffanin S, Li Y, Cornella H, Llovet JM and Russell DW (2012) Induction of hepatocellular carcinoma by in vivo gene targeting. *Proc Natl Acad Sci USA* **109**, 11264–11269.
- 5 Kulik LM and Chokechanchaisakul A (2015) Evaluation and management of hepatocellular carcinoma. *Clin Liver Dis* **19**, 23–43.
- 6 Schafer DF and Sorrell MF (1999) Hepatocellular carcinoma. *Lancet* **353**, 1253–1257.
- 7 Seitz HK and Stickel F (2007) Molecular mechanisms of alcohol-mediated carcinogenesis. *Nat Rev Cancer* **7**, 599–612.
- 8 Coussens LM and Werb Z (2002) Inflammation and cancer. *Nature* **420**, 860.
- 9 Liu F, Li H, Chang H, Wang J and Lu J (2015) Identification of hepatocellular carcinoma-associated hub genes and pathways by integrated microarray analysis. *Tumori* **101**, 206–214.
- 10 Grise F, Sena S, Bidaud-Meynard A, Baud J, Hiriart JB, Makki K, Dugot-Senant N, Staedel C, Bioulac-Sage P, Zucman-Rossi J *et al.* (2012) Rnd3/RhoE is down-regulated in hepatocellular carcinoma and controls cellular invasion. *Hepatology* **55**, 1766–1775.
- 11 Shirakami Y, Gottesman ME and Blaner WS (2012) Diethylnitrosamine-induced hepatocarcinogenesis is suppressed in lecithin:retinol acyltransferase-deficient mice primarily through retinoid actions immediately after carcinogen administration. *Carcinogenesis* **33**, 268–274.
- 12 Karakatsanis A, Papaconstantinou I, Gazouli M, Lyberopoulou A, Polymeneas G and Voros D (2013) Expression of microRNAs, miR-21, miR-31, miR-122, miR-145, miR-146a, miR-200c, miR-221, miR-222, and miR-223 in patients with hepatocellular carcinoma or intrahepatic cholangiocarcinoma and its prognostic significance. *Mol Carcinog* **52**, 297.
- 13 Yu L, Ding GF, He C, Sun L, Jiang Y and Zhu L (2014) MicroRNA-424 is down-regulated in hepatocellular carcinoma and suppresses cell migration and invasion through c-Myb. *PLoS One* **9**, e91661.
- 14 Ji C, Liu H, Yin Q, Li H and Gao H (2017) miR-93 enhances hepatocellular carcinoma invasion and metastasis by EMT via targeting PDCD4. *Biotech Lett* **39**, 1621–1629.
- 15 Guo XB, Hua Z, Li C, Peng LP, Wang JS, Wang B and Zhi QM (2015) Biological significance of long non-coding RNA FTX expression in human colorectal cancer. *Int J Clin Exp Med* **8**, 15591.
- 16 Quan M, Chen J and Zhang D (2015) Exploring the secrets of long noncoding RNAs. *Int J Mol Sci* **16**, 5467.

- 17 Zhao J, Greene CM, Gray SG and Lawless MW (2014) Long noncoding RNAs in liver cancer: what we know in 2014. *Expert Opin Ther Targets* **18**, 1207–1218.
- 18 Yu W, Qiao Y, Tang X, Ma L, Wang Y, Zhang X, Weng W, Pan Q, Yu Y, Sun F *et al.* (2014) Tumor suppressor long non-coding RNA, MT1DP is negatively regulated by YAP and Runx2 to inhibit FoxA1 in liver cancer cells. *Cell Signal* **26**, 2961–2968.
- 19 Shi SJ, Wang LJ, Yu B, Li YH, Jin Y and Bai XZ (2015) LncRNA-ATB promotes trastuzumab resistance and invasion-metastasis cascade in breast cancer. *Oncotarget* **6**, 11652–11663.
- 20 Anders S and Huber W (2010) Differential expression analysis for sequence count data. *Genome Biol* **11**, 1–12.
- 21 Benjamini Y and Hochberg Y (1995) Controlling the false discovery rate – a practical and powerful approach to multiple testing. *J Roy Stat Soc* **57**, 289–300.
- 22 Uray IP, Dmitrovsky E and Brown PH (2016) Retinoids and rexinoids in cancer prevention: from laboratory to clinic. *Semin Oncol* **43**, 49–64.
- 23 Yanagitani A, Yamada S, Yasui S, Shimomura T, Murai R, Murawaki Y, Hashiguchi K, Kanbe T, Saeki T, Ichiba M *et al.* (2004) Retinoic acid receptor alpha dominant negative form causes steatohepatitis and liver tumors in transgenic mice. *Hepatology* **40**, 366–375.
- 24 Cheng S, Prot JM, Leclerc E and Bois FY (2012) Zonation related function and ubiquitination regulation in human hepatocellular carcinoma cells in dynamic vs. static culture conditions. *BMC Genom* **13**, 54.
- 25 Yu Y, Ping J, Chen H, Jiao L, Zheng S, Han ZG, Hao P and Huang J (2010) A comparative analysis of liver transcriptome suggests divergent liver function among human, mouse and rat. *Genomics* **96**, 281–289.
- 26 Ohta K, Hoshino H, Wang J, Ono S, Iida Y, Hata K, Huang SK, Colquhoun S and Hoon DS (2015) MicroRNA-93 activates c-Met/PI3K/Akt pathway activity in hepatocellular carcinoma by directly inhibiting PTEN and CDKN1A. *Oncotarget* **6**, 3211–3224.
- 27 Blaner WS, O’Byrne SM, Wongsiriroj N, Kluwe J, D’Ambrosio DM, Jiang H, Schwabe RF, Hillman EM, Piantedosi R and Libien J (2009) Hepatic stellate cell lipid droplets: a specialized lipid droplet for retinoid storage. *Biochem Biophys Acta* **1791**, 467–473.
- 28 Revill K, Wang T, Lachenmayer A, Kojima K, Harrington A, Li J, Hoshida Y, Llovet JM and Powers S (2013) Genome-wide methylation analysis and epigenetic unmasking identify tumor suppressor genes in hepatocellular carcinoma. *Gastroenterology* **145**, 1424–1435.e1–25.
- 29 Ebert MS and Sharp PA (2010) Emerging roles for natural microRNA sponges. *Curr Biol* **20**, R858–R861.
- 30 Xia T, Liao Q, Jiang X, Shao Y, Xiao B, Xi Y and Guo J (2014) Long noncoding RNA associated-competing endogenous RNAs in gastric cancer. *Sci Rep* **4**, 6088.
- 31 Yang X, Wang CC, Lee WYW, Trovik J, Chung TKH and Kwong J (2018) Long non-coding RNA HAND2-AS1 inhibits invasion and metastasis in endometrioid endometrial carcinoma through inactivating neuromedin U. *Cancer Lett* **413**, 23–34.
- 32 Yang Y, Chen L and Gu J (2017) Recurrently deregulated lncRNAs in hepatocellular carcinoma. *Nat Commun* **8**, 14421.
- 33 Huang X, Qin J and Lu S (2015) Up-regulation of miR-877 induced by paclitaxel inhibits hepatocellular carcinoma cell proliferation through targeting FOXM1. *Int J Clin Exp Pathol* **8**, 1515–1524.
- 34 Jin H, Wang C, Jin G, Ruan H, Gu D, Wei L, Wang H, Wang N, Arunachalam E, Zhang Y *et al.* (2017) Regulator of calcineurin 1 gene isoform 4, down-regulated in hepatocellular carcinoma, prevents proliferation, migration, and invasive activity of cancer cells and metastasis of orthotopic tumors by inhibiting nuclear translocation of NFAT1. *Gastroenterology* **153**, 799–811.e33.
- 35 Li Y, Lu W, Chen D, Boohaker RJ, Zhai L, Padmalayam I, Wennerberg K, Xu B and Zhang W (2015) KIFC1 is a novel potential therapeutic target for breast cancer. *Cancer Biol Ther* **16**, 1316–1322.
- 36 Zhang C, Chen X, Chen X, Wang X, Ji A, Jiang L, Sang F and Li F (2016) miR-135a acts as a tumor suppressor in gastric cancer in part by targeting KIFC1. *Oncotargets Ther* **9**, 3555–3563.
- 37 Liu Y, Zhan P, Zhou Z, Xing Z, Zhu S, Ma C, Li Q, Zhu Q, Miao Y, Zhang J *et al.* (2016) The overexpression of KIFC1 was associated with the proliferation and prognosis of non-small cell lung cancer. *J Thorac Dis* **8**, 2911–2923.
- 38 Chen J, Li S, Zhou S, Cao S, Lou Y, Shen H, Yin J and Li G (2017) Kinesin superfamily protein expression and its association with progression and prognosis in hepatocellular carcinoma. *J Cancer Res Ther* **13**, 651–659.
- 39 Liu M, Zhang Y, Liao Y, Chen Y, Pan Y, Tian H, Zhan Y and Liu D (2015) Evaluation of the antitumor efficacy of RNAi-mediated inhibition of CDC20 and heparanase in an orthotopic liver tumor model. *Cancer Biother Radiopharm* **30**, 233–239.
- 40 Van ZW and Wolthuis RM (2010) Cyclin A and Nek2A: APC/C-Cdc20 substrates invisible to the mitotic spindle checkpoint. *Biochem Soc Trans* **38**, 72–77.
- 41 Liao Y, Shikapwashya ON, Shteyer E, Dieckgraefe BK, Hruz PW and Rudnick DA (2004) Delayed hepatocellular mitotic progression and impaired liver regeneration in early growth response-1-deficient mice. *J Biol Chem* **279**, 43107–43116.