Identification of Potential miRNAs-mRNAs Regulatory Network From CHB to HCC Based on Bioinformatic Analysis

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Abstract

Background: A hepatitis B virus (HBV) infection is one of the major risk factors of hepatocellular carcinoma (HCC) in China. Altered expression of miRNAs has been observed in tumors. Our study aimed to identify potential microRNAs (miRNA)-messenger RNAs (mRNAs) regulatory pathways contributing to the pathogenesis of HBV-related HCC.

Methods: Microarray GSE67882 was downloaded from the Gene Expression Omnibus (GEO) database. GEO2R was used to conduct differential expression analysis. Functional enrichment of differentially-expressed miRNAs (DE-miRNAs) and pathway analyses of HCC were revealed. Target genes of DE-miRNAs were predicted by miRTarBase. Furthermore, miRNA-gene networks were constructed. The prognostic roles of screened differentially-expressed miRNAs in HCC were further evaluated using the Kaplan-Meier plotter database.

Results: A total of 24 upregulated and 21 downregulated DE-miRNAs were accessed. EGFR, MYC and MDM2 may be key targets involved in the occurrence and development of HBV-related HCC whereas hsa-miR-21 and hsa-let-7f-1 may be two potential regulators involved in the occurrence and development of HBV-related HCC.

Conclusions: In this study, potential miRNA-mRNA regulatory pathways were identified and screened and hsa-miR-21 and hsa-let-7f-1 may play a significant role in the underlying pathogenesis from chronic hepatitis B (CHB) to HBV-related HCC.

1. Introduction

Several previous reports on cancer burden showed that China has a lower cancer incidence but higher cancer mortality than the United Kingdom and the United States of America, especially involving digestive tract cancers (stomach, liver, and esophagus cancer). The reasons for the higher mortality in China might be attributed to the low rate of early detection and diagnosis of cancer, and non-uniformed clinical cancer treatment strategies performed by different regions [1–2]. Reaching about 85–95% of cases of primary liver cancer, HCC can be considered as a main pathological pattern of primary liver cancer in China [3]. Hepatocarcinogenesis is a multistep process from chronic hepatitis through cirrhosis to HCC. Research showed that a HBV infection as a major risk factor contributed up to over 50% in HCC cases [4]. Hence, exploring the underlying molecular mechanism associated with hepatocarcinogenesis is essential for identifying potential therapeutic targets, particularly in HBV-related HCC.

MiRNAs are small RNAs, which are endogenous ~ 23 nucleotides in length and often negatively regulate the expression of complementary mRNAs [5–6]. Mounting evidence suggested that miRNAs have a relationship with regulate oncogenes, tumor suppressor, and several cancer-related genes, including genes involved in migration and invasion [7]. MiRNAs might play dual role during the development and progression of HCC. MiRNAs could be regarded cancer-promoting genes that prevent the normal action of tumor suppressor genes, or prevent cell progression to cancer by repressing the expression of oncogenic

targets. A wealth of supporting evidence documented that dysregulation of miRNAs is a hallmark of HCC [8]. In HCC, miRNAs such as miR-552 and miR-650 are overexpressed [9–10], whereas miR-944 and miR-645 are downregulated [11–12]. Which miRNAs might contribute to the diagnostic and prognostic values of HBV-related HCC is unclear. We aimed to identify differentially-expressed miRNAs from CHB to HBV-related HCC, which may provide valuable clues for the carcinogenesis and pathogenesis in the HBV-related liver.

2. Results

2.1 Identification of DE-miRNAs

To identify DE-miRNAs of GSE67882 downloaded from GEO, GEO2R analysis showed that a total of 45 miRNAs were found to be significantly differentially expressed in HBV-related HCC tumor tissue when compared to CHB liver tissue, including 24 upregulated miRNAs and 21 downregulated miRNAs. A volcano plot of these DE-miRNAs is shown in Fig. 1. For better visualization, the top ten most upregulated miRNAs and the top ten most downregulated miRNAs are presented in Table 1A and Table 1B.

Table.1A Top ten upregulated differentially expressed miRNAs between tumor tissue of HBV-related HCC and liver tissue of CHB

miRNA	Log ₂ FC	Adj.Pval	P.value	t	B
hsa-miR-29b	3.65926	0.000437	0.00000182	7.57182	5.375
hsa-miR-142-3p	3.05488	0.07674	0.00262728	3.6084	-1.691
hsa-miR-21	3.03907	0.006681	0.00005567	5.57401	2.069
hsa-miR-210	2.48626	0.020936	0.0002617	5.13017	0.719
hsa-miR-106b	2.15617	0.07674	0.00253717	3.67749	-1.612
hsa-miR-146b-5p	2.05892	0.07674	0.00319749	3.51208	-1.88
hsa-miR-374a	1.86331	0.174395	0.02189598	2.63806	-3.571
hsa-let-7i	1.82453	0.032305	0.00059769	4.34128	-0.251
hsa-miR-29c	1.81177	0.084509	0.00452451	3.34196	-2.215
hsa-miR-29a	1.76615	0.07674	0.00303683	3.53735	-1.831

Table.1B Top ten downregulated differentially expressed miRNAs between tumor tissue of HBV related HCC and liver tissue of CHB

miRNA	Log ₂ FC	Adj.Pval	P.value	t	B
hsa-miR-425*	-2.42576	0.214055	0.03300018	-2.58495	-3.474
hsa-let-7f-1*	-2.31608	0.257235	0.04715973	-2.35345	-3.796
hsa-miR-1274a	-2.27009	0.246717	0.04420344	-2.19886	-4.355
hsa-miR-378*	-2.21661	0.032305	0.00067302	-4.56702	-0.18
hsa-miR-1260	-2.20806	0.174395	0.02252596	-2.54623	-3.736
hsa-miR-1274b	-1.99441	0.226579	0.03784977	-2.28001	-4.214
hsa-miR-720	-1.92966	0.154758	0.01741033	-2.67606	-3.496
hsa-miR-194*	-1.67749	0.084509	0.00501651	-3.43831	-2.05
hsa-miR-892b	-1.66462	0.226579	0.03761873	-2.36845	-3.897

2.2 Identification of target genes of the selected DE-miRNAs

Based on the fold change, hsa-miR-29b, hsa-miR-142-3p, and hsa-miR-21 were the top three upregulated miRNAs, while hsa-miR-425, hsa-let-7f-1, and hsa-miR-1274a were the top three downregulated miRNAs. These selected DE-miRNAs were selected for further analysis. Using the miRTarBase, 169 potential target genes of the top three down regulated miRNAs and 1250 target genes of the top three upregulated miRNAs were predicted.

2.3 GO term functional enrichment analysis

GO functional annotation of target genes of the selected DE-miRNA was performed using the DAVID database. The results indicated that changes in the BP of 1250 target genes were mainly enriched in positive regulation of the macromolecule metabolic process, skeletal system development, regulation of apoptosis, extracellular matrix organization, and regulation of programmed cell death (Table 2). Changes in MF were significantly enriched in platelet-derived growth factor binding, platelet-derived growth factor receptor binding, extracellular matrix structural constituent, protein dimerization activity, and cell surface binding (Table 2). Changes in the CC of 1250 target genes of the selected DE-miRNAs were mainly enriched in the extracellular matrix part, collagen, basement membrane, extracellular matrix, and proteinaceous extracellular matrix (Table 2). Furthermore, 169 potential target genes of downregulated miRNAs were significantly enriched in the poly(A) RNA binding of poly(A) RNA, DNA, protein, transcription factor in the MF category, nucleus, nucleoplasm, cytosol, nuclear chromosome, telomeric region, catalytic step 2 spliceosome in the CC category and transcription, DNA-templated, regulation of transcription, response to drugs, DNA-templated, and response to glucose in the BP category (Table 2).

Table.2 GO annotation for the target genes of the selected DE-miRNAs. (Top 3 enriched MF, CC and BP for target genes of upregulated and downregulated DE-miRNAs)

Category	Term	Pathway description	Count in gene set	P-Value
Upregulated miRNAs				
MF term	GO:0048407	platelet-derived growth factor binding	9	7.62E-10
MF term	GO:0005161	platelet-derived growth factor receptor binding	7	1.35E-06
MF term	GO:0005201	extracellular matrix structural constituent	13	1.21E-04
MF term	GO:0046983	protein dimerization activity	39	3.44E-04
MF term	GO:0043498	cell surface binding	7	7.60E-04
CC term	GO:0044420	extracellular matrix part	25	1.58E-12
CC term	GO:0005581	collagen	15	3.85E-12
CC term	GO:0005604	basement membrane	18	1.10E-09
CC term	GO:0031012	extracellular matrix	36	1.37E-08
CC term	GO:0005578	proteinaceous extracellular matrix	33	8.19E-08
BP term	GO:0010604	positive regulation of macromolecule metabolic process	71	2.13E-09
BP term	GO:0001501	skeletal system development	36	2.95E-08
BP term	GO:0042981	regulation of apoptosis	64	7.04E-08
BP term	GO:0030198	extracellular matrix organization	19	9.18E-08
BP term	GO:0043067	regulation of programmed cell death	64	9.98E-08
Downregulated miRNAs				
MF term	GO:0044822	poly(A) RNA binding	24	2.57E-04
MF term	GO:0003677	DNA binding	31	2.62E-04
MF term	GO:0005515	protein binding	101	0.001086967
MF term	GO:0003700	transcription factor activity, sequence-specific DNA binding	20	0.001308091
MF term	GO:0008134	transcription factor binding	9	0.004764204
CC term	GO:0005634	nucleus	81	1.45E-07

Category	Term	Pathway description	Count in gene set	P-Value
CC term	GO:0005654	nucleoplasm	48	7.13E-06
CC term	GO:0005829	cytosol	44	0.005899698
CC term	GO:0000784	nuclear chromosome, telomeric region	6	0.006285841
CC term	GO:0071013	catalytic step 2 spliceosome	5	0.009370925
BP term	GO:0006351	transcription, DNA-templated	38	1.91E-05
BP term	GO:0045892	negative regulation of transcription, DNA-templated	15	2.51E-04
BP term	GO:0042493	response to drug	11	5.87E-04
BP term	GO:0006355	regulation of transcription, DNA-templated	27	0.001582013
BP term	GO:0009749	response to glucose	5	0.003765161

2.4 KEGG pathway enrichment analysis

KEGG pathway enrichment analysis was conducted by the Enrichr database. The enriched KEGG pathway for 1250 target genes of upregulated miRNAs were mainly enriched in pathways in cancer, small cell lung cancer (SCLC), the PI3K-Akt signaling pathway, hepatitis B, AGE-RAGE signaling pathway in diabetic complications, microRNAs in cancer, prostate cancer, human papillomavirus infection, focal adhesion, and pancreatic cancer (Fig. 2A). However, the 169 target genes of downregulated miRNAs were mainly enriched in salmonella infection, Cushing syndrome, circadian rhythm, B cell receptor signaling pathway, adherens junction, cell cycle, vasopressin-regulated water reabsorption, colorectal cancer, Fc gamma R-mediated phagocytosis, and prostate cancer (Fig. 2B). Obviously, both target genes of dysregulated miRNAs were enriched in focal adhesion and prostate cancer, which deserved be further studied. The cluster diagram of target genes in the KEGG pathway is presented in Fig. 3A and Fig. 3B.

2.5 PPI network and hub genes of the DE-miRNA network construction and analysis

The PPI network of target genes of selected DE-miRNAs was predicted by STRING and constructed by Cytoscape. Then, the top 10 genes with the highest degree of PPI network connectivity were identified as hub genes using CytoHubba (Fig. 4A and Fig. 4B). The names and abbreviations of the top 10 genes are shown in Table 3. For upregulated miRNAs, the top 10 hub genes of distinction between HBV-related HCC tumor samples with CHB liver samples were identified, including MYC, PTEN, CTNNB1, IL6, VEGFA, STAT3, CCND1, CDC42, ESR1, and MMP2. For downregulated miRNAs, the top 10 hub genes were MDM2, HNRNPM, RAC1, GSK3B, CDKN1B, HIST1H4C, HNRNPU, SMAD2, CREB1, and PPIL3. Based on the node

degree of hub genes, EGFR and MDM2 may be two key targets involved in the occurrence and development of HBV-related HCC.

2.6 Analysis of the overlapped genes of dysregulated target miRNAs (hsa-miR-21 and hsa-let-7f-1) and hub genes network

Using a Venn diagram, we found that seven (PTEN, STAT3, CDC42, VEGFA, MYC, CCND1, and EGFR) in ten hub genes could be potentially modulated by upregulated hsa-miR-21, while six genes (PTEN, STAT3, CDC42, VEGFA, MYC, and ESR1) and three genes (IL6, CTNNB1, and ESR1) were modulated by has-miR-29b and hsa-miR-142-3p, respectively. For downregulated DE-miRNAs, hsa-let-7f-1 could potentially target eight (MDM2, HNRNPM, GSK3B, CDKN1B, HNRNPU, SMAD2, CREB1, and PPIL3) in ten hub genes, whereas two hub genes (RAC1 and HIST1H4C) could be potentially modulated by hsa-miR-425 (Fig. 5A and Fig. 5B). Therefore, hsa-miR-21 and hsa-let-7f-1 might be two potential regulators involved in the occurrence and development of HBV-related HCC.

Furthermore, we evaluated the expression of these overlapped genes in HCC using the UALCAN database. We found that the expression of PTEN, CDC42 and VEGFA was markedly upregulated in HCC tissue compared to normal tissue whereas the MYC gene was significantly downregulated (Fig. 6a-g). It is widely acknowledged that there is an inverse relationship between miRNA expression and target gene expression [6]. Thus, among these detected genes, the significantly downregulated MYC gene may be the most potential target for the upregulated hsa-miR-21. Theoretically, the significantly upregulated genes, including MDM2, HNRNPM, GSK3B, CDKN1B, HNRNPU, SMAD2, CREB1, and PPIL3 may be the most potential targets for the downregulated hsa-let-7f-1 (Fig. 6h-o). Combined, these findings indicated that upregulated hsa-miR-21 and downregulated hsa-let-7f-1 may promote the initiation and progression of HBV-related HCC.

To go a step further and validate this conclusion, the prognostic values of hsa-miR-21 and hsa-let-7f-1 were obtained using the Kaplan-Meier plotter database (Fig. 7). On the one hand, the Kaplan-Meier curve suggested that high expression of has-miR-21 as a tumor promoter has poorer prognosis ($P = 0.0055$), further implying that upregulated has-miR-21 may not only promote the initiation and progression from CHB to HBV-related HCC, but may also predict worse outcomes in HCC. On the other hand, downregulated hsa-let-7f-1 played a worse prognostic role in HCC ($P = 0.024$). Therefore, aberrant expression of has-miR-21 and hsa-let-7f-1 can be considered two prognostic biomarkers.

Table.3 The degree of top 10 hub genes identified in the PPI interaction

Upregulated miRNAs			Downregulated miRNAs		
Gene symbol	Gene description	Degree	Gene symbol	Gene description	Degree
EGFR	epidermal growth factor receptor	201	MDM2	MDM2 proto-oncogene	14
MYC	MYC proto-oncogene	189	HNRNPM	heterogeneous nuclear ribonucleoprotein M	11
PTEN	phosphatase and tensin homolog	161	RAC1	Rac family small GTPase 1	11
IL6	Interleukin 6	156	GSK3B	glycogen synthase kinase 3 beta	10
VEGFA	vascular endothelial growth factor A	153	CDKN1B	cyclin dependent kinase inhibitor 1B	10
STAT3	signal transducer and activator of transcription 3	146	HIST1H4C	H4 clustered histone 3	9
CTNNB1	catenin beta 1	145	HNRNPU	heterogeneous nuclear ribonucleoprotein U	8
CCND1	cyclin D1	127	SMAD2	SMAD family member 2	8
ESR1	estrogen receptor 1	127	CREB1	cAMP responsive element binding protein 1	8
CDC42	cell division cycle 42	122	PPIL3	peptidylprolyl isomerase like 3	7

3. Discussion

In this study, we aimed to explore the occurrence and progression of HBV-related HCC at the miRNA level by performing differential expression analysis using a miRNA array downloaded from the GEO datasets. Compared with CHB liver tissue, overexpression of 24 miRNAs and low expression of 21 miRNAs were found to be differentially expressed in HVB-related HCC. Using the miRTarBase, potential target genes (1250 and 169 respectively) of the top three upregulated and downregulated miRNAs were predicted. According to the node degree, the top ten hub genes of dysregulated miRNAs were screened out. Among

these genes, EGFR, MYC, and MDM2 demonstrated the highest degrees, which were 201, 189, and 14, respectively (Table 3). Based on the overlap of hub genes and genes of dysregulated target miRNAs, we found that most hub genes were mainly modulated by upregulated has-miR-21 and downregulated has-let-7f-1, respectively. Therefore, the most potential regulators (hsa-miR-21 and hsa-let-7f-1) involved in the occurrence and development of HBV-related HCC were screened.

In our study, KEGG pathway analysis revealed that target genes of differentially-expressed miRNAs were mainly enriched in various types of cancer (SCLC, prostate cancer, pancreatic cancer, colorectal cancer, etc.), focal adhesion, and the PI3K-Akt signaling pathway. Akt as a serine-threonine kinase is directly activated by PI3K to promote cancer cell growth, proliferation, and apoptosis survival [20]. The phosphatidylinositol-3-kinase/protein kinase B(PI3K-AKT) signaling pathway, a dysregulated pathway in HCC, was vital for the growth and survival of cancer and was associated with poor survival [19–21]. Moreover, the target genes enriched in focal adhesion formation were closely related to invasion and metastases of human cancer cells [6,22]. Thus, the differentially-expressed miRNAs of the PI3K-AKT signaling pathway, focal adhesion and cancer-related pathway, which were determined by KEGG analysis may be associated with the development of HBV-related HCC.

In our study, we found that overexpressed hsa-miR-21 was involved in the occurrence, development, and poorer prognosis of HBV-related HCC. Our findings therefore support prior observations that miR-21 was highly overexpressed and mediated phenotypic characteristics in HCC, prostate cancer, and clear-cell kidney cancer [23–25]. Thus, mir-21, which was significantly upregulated in HBV-related HCC, may be a tumorigenic factor miRNA.

Our findings supported that low expressed has-let-7f-1 may promote oncogenesis from CHB to HCC. This result is roughly consistent with the consensus from the previous observation. In a previous study, it was reported that the human let-7 family miRNA as candidate tumor suppressors have been directly implicated in cancer, including lung tumor and endocervical epithelial cells [26–27]. Takamizawa et al. presented the first evidence of the reduced expression of the let-7 microRNA in human lung cancers, indicating shortened postoperative survival [27]. To sum up, it is extremely meaningful to authenticate the functions of hsa-miR-21 and hsa-let-7f-1 in HCC and elucidate the underlying mechanisms from CHB changing into HBV-related HCC.

Because serum miRNAs can indicate the level of tissue miRNAs, serum miRNAs are likely to be potential biomarkers, such as alpha fetoprotein (AFP) for HCC. Wu et al. proved that serum miR-4651 might be a useful biomarker for aflatoxin B1-positive HCC and Zekri et al. suggested that combining two serum miRNAs (miR-22 and miR-199a-3p) with AFP can identify HCC in patients with chronic hepatitis C [28–29]. Moreover, the expression level of serum miRNA-27a and miRNA-18b are potential diagnostic biomarkers in differentiating HCC from chronic hepatitis C patients [30]. In a previous study, it was revealed that changes in serum miR-21 were observed earlier and more accurately reflected the pathogenesis of HCC than AFP in an animal model [31]. In our study, upregulated hsa-miR-21 and

downregulated hsa-let-7f-1 were involved in carcinogenesis and the pathogenesis of HBV-related HCC, the expression level of these two miRNAs might act as discriminators between HCC and CHB.

4. Conclusion

In conclusion, we have successfully identified upregulated has-miR-21 and down regulated has-let-7f-1 as potential predictors for assessing the prognosis in HBV-related HCC based on bioinformatic analysis. According to the KEGG pathway enrichment analysis and Kaplan-Meier plotter, has-miR-21 and has-let-7f-1 might participate in the onset and migration of HBV-related HCC leading to poor prognosis. It is worth to further study the underlying molecular mechanism of these two miRNAs in HBV-related HCC.

5. Materials And Methods

5.1 MicroRNA microarray

First, we obtained miRNAs that were expressed in the CHB group (control group) and HBV-related HCC group (experimental group) from the v) based on the keywords “hepatocellular carcinoma”, “hepatitis B virus” and “homo sapiens”. Next, we independently assessed the titles and abstracts identified from the search strategy. Moreover, we preferred GEO series based on clinical liver tissues rather than a cell line. Eventually, only the dataset GEO series named GSE67882 was retrieved. The dataset GSE67882 was retrieved from the platform of GPL10850 Agilent-021827 Human miRNA Microarray (V3) (miRBase release 12.0 miRNA ID version), and contained four HBV-related HCC tumor tissues and eight CHB liver tissues.

5.2 Data processing and screening of candidate DE-miRNA

To screen differentially-expressed miRNAs (DE-miRNAs) between HBV-related HCC tumor tissue and liver tissue of CHB. The DE-miRNAs of GSE67882 were obtained by an GEO2R online tool (<https://www.ncbi.nlm.nih.gov/geo/geo2r>)[13]. Screening of DE-miRNAs between HBV-related HCC tumor tissue and non-tumor liver tissue of CHB as well as volcano mapping were performed using SangerBox. Furthermore, genes that met the statistically significant criteria of $|\log_2 \text{FC (fold change)}| \geq 1.0$ and $P\text{-value} < 0.05$ were considered DE-miRNAs.

5.3 Prediction target genes of potential DE-miRNAs

The potential target genes of the top three most upregulated and downregulated miRNAs were predicted by miRTarBase (<http://mirtarbase.mbc.nctu.edu.tw/php/index.php>), which is an experimentally validated miRNA-target interactions database [14].

5.4 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for the

predicted targets of selected DE-miRNAs

To perform functional annotation, the DAVID online database (version 6.8) (<http://david.ncifcrf.gov>) for annotation, visualization, and integrated discovery was introduced. GO is a major bioinformatics tool to annotate genes and analyze biological functions, which consists of three parts, including molecular function (MF), cellular component (CC) and biological process (BP). Moreover, the Enrichr database (<http://amp.pharm.mssm.edu/Enrichr/>) was used to perform KEGG pathway enrichment analysis for the predicted targets of the selected DE-miRNAs. KEGG is a database resource for understanding high-level functions and biological systems from large-scale molecular datasets generated by high-throughput experimental technologies. P-value < 0.05 was considered statistically significant.

5.5 PPI network and miRNA-gene network construction

The protein-protein interaction (PPI) network of target genes was predicted by the Search Tool for the Retrieval of Interacting Genes (STRING; <http://string-db.org/>) (version 11.0) online database [15]. Furthermore, PPI networks were constructed using Cytoscape software (version 3.8.0) [16]. Subsequently, using the CytoHubba plugin in Cytoscape, the top 10 nodes with the highest degree of PPI network connectivity were identified as hub genes. Finally, the Venn diagram web tool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) was constructed to identify the selected DE-miRNAs and hub genes network.

5.6 Analysis of overlapped genes of the dysregulated target miRNAs and hub genes network

We used the UALCA database as a web server for analyzing the expression of target genes from the Cancer Genome Atlas (TCGA) between HBV-related HCC tumor tissues when compared to CHB liver tissues, including differential expression analysis and patient survival analyses [17].

5.7 Survival data from the Kaplan–Meier plotter

The prognostic values of hsa-miR-21 and hsa-let-7f-1 in HCC were analyzed using the Kaplan–Meier plotter database (<http://kmplot.com/analysis/>) [18]. P < 0.05 was considered statistically significant.

Abbreviations

HCC: hepatocellular carcinoma; MiRNA: MicroRNA; DE-miRNAs: differentially expressed miRNA; mRNA: messenger RNAs; PI3K-AKT: phosphatidylinositol-3-kinase/protein kinase B; GEO: Gene Expression Omnibus; PPI: protein-protein interaction network; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: biological process; CC: cellular component; MF: molecular function; TCGA: the Cancer Genome Atlas; MCODE: Molecular Complex Detection; CHB: chronic hepatitis B; OS: overall survival; SCLC: small cell lung cancer; AFP: alpha fetoprotein.

Declarations

Author disclosure statement

- Ethical Approval and Consent to participate

There is no ethical approval and consent to participate because our work is base on bioinformatic analysis.

- Availability of data and materials

All data generated or analysed during this study are included in this published article

- Competing interests

The authors report no conflicts of interest in this work.

- Funding

The authors have not received a fee from any organization to write this article.

- Authors' contributions

Li Bai contributed to the conception of the study;

Xindan Kang contributed significantly to analysis and manuscript preparation;

Xin Shu performed the data analyses and wrote the manuscript;

Fangbin Hao helped perform the analysis with constructive discussions.

References

1. Ayyar, K.K., and Reddy, K.V.R. (2018). MAPK and NF- κ B signalling pathways regulate the expression of miRNA, let-7f in human endocervical epithelial cells. *J Cell Biochem* 119(6), 4751-4759. doi: 10.1002/jcb.26665.
2. Bartel, D.P. (2009). MicroRNAs: target recognition and regulatory functions. *Cell* 136(2), 215-233. doi: 10.1016/j.cell.2009.01.002.
3. Chandrashekar, D.S., Bashel, B., Balasubramanya, S.A.H., Creighton, C.J., Ponce-Rodriguez, I., Chakravarthi, B., et al. (2017). UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia* 19(8), 649-658. doi: 10.1016/j.neo.2017.05.002.
4. Chen, W., Zheng, R., Baade, P.D., Zhang, S., Zeng, H., Bray, F., et al. (2016). Cancer statistics in China, 2015. *CA Cancer J Clin* 66(2), 115-132. doi: 10.3322/caac.21338.
5. Chen, W.Q., Li, H., Sun, K.X., Zheng, R.S., Zhang, S.W., Zeng, H.M., et al. (2018a). Cancer Incidence and Mortality in China, 2014. *Chinese journal of cancer research* 40(1), 5-13. doi: 10.3760/cma.j.issn.0253-3766.2018.01.002.

6. Chen, Z., Zhou, Z.Y., He, C.C., Zhang, J.L., Wang, J., and Xiao, Z.Y. (2018b). Down-regulation of LncRNA NR027113 inhibits cell proliferation and metastasis via PTEN/PI3K/AKT signaling pathway in hepatocellular carcinoma. *Eur Rev Med Pharmacol Sci* 22(21), 7222-7232. doi: 10.26355/eurev_201811_16256.
7. Chou, C.H., Shrestha, S., Yang, C.D., Chang, N.W., Lin, Y.L., Liao, K.W., et al. (2018). miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. *Nucleic Acids Res* 46(D1), D296-d302. doi: 10.1093/nar/gkx1067.
8. Feng, R.M., Zong, Y.N., Cao, S.M., and Xu, R.H. (2019). Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? *Cancer Commun (Lond)* 39(1), 22. doi: 10.1186/s40880-019-0368-6.
9. Gao, P., Wong, C.C., Tung, E.K., Lee, J.M., Wong, C.M., and Ng, I.O. (2011). Deregulation of microRNA expression occurs early and accumulates in early stages of HBV-associated multistep hepatocarcinogenesis. *J Hepatol* 54(6), 1177-1184. doi: 10.1016/j.jhep.2010.09.023.
10. Guo, H., Ingolia, N.T., Weissman, J.S., and Bartel, D.P. (2010). Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature* 466(7308), 835-840. doi: 10.1038/nature09267.
11. Han, L.L., Yin, X.R., and Zhang, S.Q. (2018). miR-650 Promotes the Metastasis and Epithelial-Mesenchymal Transition of Hepatocellular Carcinoma by Directly Inhibiting LATS2 Expression. *Cell Physiol Biochem* 51(3), 1179-1192. doi: 10.1159/000495495.
12. Juan, D., Alexe, G., Antes, T., Liu, H., Madabhushi, A., Delisi, C., et al. (2010). Identification of a microRNA panel for clear-cell kidney cancer. *Urology* 75(4), 835-841. doi: 10.1016/j.urology.2009.10.033.
13. Kumar, G., Kumar, R., Pal, M.K., Pramanik, N., Lahiri, T., Gupta, A., et al. (2021). APT: An Automated Probe Tracker From Gene Expression Data. *IEEE/ACM Trans Comput Biol Bioinform* 18(5), 1864-1874. doi: 10.1109/tcbb.2019.2958345.
14. Levrero, M., and Zucman-Rossi, J. (2016). Mechanisms of HBV-induced hepatocellular carcinoma. *J Hepatol* 64(1 Suppl), S84-s101. doi: 10.1016/j.jhep.2016.02.021.
15. Li, C., Wang, Z., Chen, S., Zhang, J., Qu, K., and Liu, C. (2018). MicroRNA-552 promotes hepatocellular carcinoma progression by downregulating WIF1. *Int J Mol Med* 42(6), 3309-3317. doi: 10.3892/ijmm.2018.3882.
16. Lou, W., Chen, J., Ding, B., Chen, D., Zheng, H., Jiang, D., et al. (2018). Identification of invasion-metastasis-associated microRNAs in hepatocellular carcinoma based on bioinformatic analysis and experimental validation. *J Transl Med* 16(1), 266. doi: 10.1186/s12967-018-1639-8.
17. Lv, L., Wang, X., and Ma, T. (2019). microRNA-944 inhibits the malignancy of hepatocellular carcinoma by directly targeting IGF-1R and deactivating the PI3K/Akt signaling pathway. *Cancer Manag Res* 11, 2531-2543. doi: 10.2147/cmar.S199818.
18. Nagy, Á., Lánckzy, A., Menyhárt, O., and Györffy, B. (2018). Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. *Sci Rep* 8(1), 9227. doi: 10.1038/s41598-018-27521-y.

19. Rashad, N.M., El-Shal, A.S., Shalaby, S.M., and Mohamed, S.Y. (2018). Serum miRNA-27a and miRNA-18b as potential predictive biomarkers of hepatitis C virus-associated hepatocellular carcinoma. *Mol Cell Biochem* 447(1-2), 125-136. doi: 10.1007/s11010-018-3298-8.
20. Ribas, J., Ni, X., Haffner, M., Wentzel, E.A., Salmasi, A.H., Chowdhury, W.H., et al. (2009). miR-21: an androgen receptor-regulated microRNA that promotes hormone-dependent and hormone-independent prostate cancer growth. *Cancer Res* 69(18), 7165-7169. doi: 10.1158/0008-5472.Can-09-1448.
21. Smoot, M.E., Ono, K., Ruscheinski, J., Wang, P.L., and Ideker, T. (2011). Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics* 27(3), 431-432. doi: 10.1093/bioinformatics/btq675.
22. Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., et al. (2015). STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res* 43(Database issue), D447-452. doi: 10.1093/nar/gku1003.
23. Takamizawa, J., Konishi, H., Yanagisawa, K., Tomida, S., Osada, H., Endoh, H., et al. (2004). Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res* 64(11), 3753-3756. doi: 10.1158/0008-5472.Can-04-0637.
24. Tao, J., Liu, Z., Wang, Y., Wang, L., Yin, G., Yang, W., et al. (2019). MicroRNA-645 represses hepatocellular carcinoma progression by inhibiting SOX30-mediated p53 transcriptional activation. *Int J Biol Macromol* 121, 214-222. doi: 10.1016/j.ijbiomac.2018.10.032.
25. Wang, X., Zhang, J., Zhou, L., Lu, P., Zheng, Z.G., Sun, W., et al. (2015). Significance of serum microRNA-21 in diagnosis of hepatocellular carcinoma (HCC): clinical analyses of patients and an HCC rat model. *Int J Clin Exp Pathol* 8(2), 1466-1478.
26. Wu, J.H., Tian, X.Y., An, Q.M., Guan, X.Y., and Hao, C.Y. (2018). LINC00963 promotes hepatocellular carcinoma progression by activating PI3K/AKT pathway. *Eur Rev Med Pharmacol Sci* 22(6), 1645-1652. doi: 10.26355/eurrev_201803_14574.
27. Wu, X.M., Xi, Z.F., Liao, P., Huang, H.D., Huang, X.Y., Wang, C., et al. (2017). Diagnostic and prognostic potential of serum microRNA-4651 for patients with hepatocellular carcinoma related to aflatoxin B1. *Oncotarget* 8(46), 81235-81249. doi: 10.18632/oncotarget.16027.
28. Xie, Y., Yao, Q., Butt, A.M., Guo, J., Tian, Z., Bao, X., et al. (2014). Expression profiling of serum microRNA-101 in HBV-associated chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. *Cancer Biol Ther* 15(9), 1248-1255. doi: 10.4161/cbt.29688.
29. Xu, F., Zhang, J., Hu, G., Liu, L., and Liang, W. (2017). Hypoxia and TGF- β 1 induced PLOD2 expression improve the migration and invasion of cervical cancer cells by promoting epithelial-to-mesenchymal transition (EMT) and focal adhesion formation. *Cancer Cell Int* 17, 54. doi: 10.1186/s12935-017-0420-z.
30. Zekri, A.N., Youssef, A.S., El-Desouky, E.D., Ahmed, O.S., Lotfy, M.M., Nassar, A.A., et al. (2016). Serum microRNA panels as potential biomarkers for early detection of hepatocellular carcinoma on top of HCV infection. *Tumour Biol* 37(9), 12273-12286. doi: 10.1007/s13277-016-5097-8.

31. Zhu, P., Liu, Z., Zhou, J., and Chen, Y. (2019). Tanshinol inhibits the growth, migration and invasion of hepatocellular carcinoma cells via regulating the PI3K-AKT signaling pathway. *Onco Targets Ther* 12, 87-99. doi: 10.2147/ott.S185997.

Figures

Figure 1

Volcano plot of the DE-miRNAs were performed using SangerBox. (the red dots and green dots represent the upregulated and downregulated miRNAs in HBV related HCC tumor samples compared with that of CHB liver samples)

A **B**

Figure 2

KEGG pathway enrichment analysis of target genes of the top three dysregulated DE-miRNA using Enrichr database. (A. pink for target genes of the top three upregulated miRNAs; B. blue for target genes of the top three downregulated miRNAs;)

Figure 3

the clustergram of target genes in KEGG pathway by Enrichr database. (A. for the target genes of the top three upregulated miRNAs; B. for the target genes of the top three downregulated miRNAs)

Figure 4

The top 10 hub genes of the selected DE-miRNA identified in the PPI interaction (A. the top 10 hub genes of the upregulated miRNA; B. the top 10 hub genes of the downregulated miRNA)

Figure 5

Venn diagram showed an overlap of target genes of dysregulated miRNAs and hub genes. (A. For upregulated miRNAs; B. For downregulated miRNAs)

Figure 6

The mRNA expression of predicted targets of hsa-miR-21 and hsa-let-7f-1 from the UALCAN database. (a)PTEN expression (P=2.56E-03); (b)STAT3 expression (P= 4.25E-01); (c)CDC42 expression (P<1E-12); (d)VEGFA expression (P<1E-12); (e)EGFR expression (P=2.49E-01); (f)CCND1 expression (P=1.791770E-01); (g)MYC expression (P=2.42E-03); (h)MDM2 expression (P=4.99E-06); (i)HNRNPM expression (P<1E-12); (j)GSK3B expression (P<1E-12); (k)CDKN1B expression (P=1.69E-12); (l) HNRNPU expression (P=1.62E-12); (m)SMAD2 expression (P=1.62E-12); (n)CREB1 expression (P<1E-12); (o)PPIL3 expression (P<1E-12).

Figure 7

The prognostic roles of hsa-miR-21 and hsa-let-7f-1 in HCC (A. Kaplan–Meier survival curve of hsa-miR-21 in HCC; B: Kaplan–Meier survival curve of hsa-let-7f-1 in HCC)