European Review for Medical and Pharmacological Sciences 2014; 18: 108-116

Identification of typical miRNAs and target genes in hepatocellular carcinoma by DNA microarray technique

T.-L. HE, K.-L. ZHENG, G. LI, B. SONG, Y.-J. ZHANG

Department of General Surgery, Changhai Hospital, Shanghai, China
Tianlin He and *Kailian Zheng* are regarded as co-first author

Abstract. – OBJECTIVES: The purpose of this study was to identify featured miRNAs of hepatocellular carcinoma (HCC) by comparing normal and cancer cell line samples and find potential utility as biomarkers for early diagnosis and treatment of HCC.

MATERIALS AND METHODS: We downloaded the gene expression profile GSE41077 from Gene Expression Omnibus database which included 6 HCC cell lines samples and 2 controls. Differentially expressed miRNAs were identified by multtest package in R language after the data normalization. The selected differentially expressed miRNAs were further analyzed using bioinformatics methods. Target genes of these miRNAs were predicted using miRTarBase and miRecords databases. STRING software was used to construct the interaction network of target genes. Finally, we made module analysis by using Cytoscape software and its plugins – MCODE and BiNGO.

RESULTS: A total of 40 differentially expressed miRNAs were identified and the remarkably down-regulated miRNA was hsa-miR-122 which included 29 high confident target genes. The interaction network of target genes was constructed among 629 interaction pairs. Four functional modules in the network were obtained, from which EGLN3, ALDOA, NCAM1 and AACS were the high confident target genes, respectively. Genes in the modules most related to biological functions of signal transmission, regulation of macromolecule metabolic process.

CONCLUSIONS: Low level of expression of hsa-miR-122 in HCC cell line is consistent with the existed previous studies. It is not only confirm the importance role of such miRNA in HCC cells, but also provide important help in identifying specific biomarker of HCC cells.

Key Words:
Hepatocellular carcinoma, Differentially expressed miRNA, Target gene, Interaction network.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third cause of cancer-related mortality worldwide[1]. More than 600,000 people die from HCC each year[2]. HCC normally develops as a consequence of underlying liver disease and is often associated with cirrhosis[3]. It is difficult to diagnose HCC in its early stages. Besides, prognosis remains poor because of tumor recurrence or tumor progression, and currently there are no well-established effective adjuvant therapies. Therefore, there is an urgent need to understand the molecular carcinogenic mechanism of HCC.

MicroRNAs (miRNAs), a class of short noncoding RNAs that regulate posttranscriptional gene expression of their target genes, constitute one of the largest classes of gene regulators in animal genome[4]. To date, more than 17,000 distinct mature miRNA sequences have been identified from over 140 species[5]. They are associated with the control of a broad range of biological processes, including development, differentiation, metabolism, cell cycle and aging[6-8]. Some of these were shown to be functionally involved in carcinogenesis and tumor progression, suggesting that miRNAs can serve as novel molecular targets for cancer therapy.

A number of recent studies have proved the involvement of miRNAs in HCC in tumor progression and metastasis[9-12]. However, there is still a need to identify new miRNAs as diagnostic biomarkers for HCC. In our study, we investigated the miRNA expression profiles between normal and cancer samples by microarray analysis. In order to further explore the molecular mechanism, we tested the transcriptional control model of miRNA emergence in HCC, predicted target genes of differentially expressed miRNAs and their mutually

Corresponding Author: Yijie Zhang, MD; e-mail: yijie06@medmail.com.cn

interacted genes, built international network and identified the functional modules from the network. Our results may provide theoretical foundation for early diagnosis and treatment of HCC.

Materials and Methods

Microarray Data

The genechip data of GSE41007[13] was obtained from National Center of Biotechnology Information Gene Expression Omnibus (GEO) database, which was based on Agilent-021827 Human miRNA Microarray V3 platform (miRBase release 12.0 miRNA ID version). Total 8 genechips from 2 normal samples and 6 samples of HCC cell lines, were available for analysis. The annotation information of all probe sets which was provided by Affymetrix Company was downloaded with the raw data file.

Data Preprocessing and Differential miRNAs Analysis

Firstly, robust multiarray average (RMA) algorithm of R language was employed to convert probe-level data in CEL files into expression measures[14] . The expression profile data were normalized by taking the average expression values. Then LIMMA package in R language[15] was used to identify differentially expressed miRNAs between the normal and cancer samples. The method of Benjamini-Hochberg (BH) in Multtest package was used to conduct multi-test and adjust the raw *p*-values into false discovery rate (FDR). The FDR less than 0.05 and |logFC| more than 1 were used as the cut-off criteria and the most significantly differentially expressed miRNAs were identified.

Hierarchical Clustering of Differentially Expressed miRNAs

The gene expression level of the same tissue was significantly different in various disease states because of the specificity of gene expression in the same species under different conditions. To intuitively observe difference of gene expression between normal and HCC cell lines, the expression values in each group were hierarchical clustered by Cluster[16].

Identifying Target Genes of the Differentially Expressed miRNA

MiRecords, an integrated resource for microRNA-target interactions, includes 1135 records of validated miRNA-target interactions between 301 miRNAs and 902 target genes in seven animal species[17]. MiRTarBase, a database curates experimentally validated microRNA-target interactions, curates 3576 experimentally verified miRNA-target interactions between 657 miRNAs and 2297 target genes among 17 species[18].

To obtain more reliable target genes, the two databases were combined with each other to retrieve the target genes of differentially expressed miRNAs with the default parameters and the ones both in these two databases were extracted.

Construction of miRNA-target Genes Interactions Network

The online database resource Search Tool for the Retrieval of Interacting Genes (STRING), which provided uniquely comprehensive coverage and ease of access to both experimental and predicted interaction information[19], was used to search functional interaction of differentially expressed genes and construct interaction networks by calculating their confidence score.

Functional Modules of Interaction Networks Analysis

Cytoscape platform was employed to process biological network visualization and data integration[20]. MCODE (Molecular Complex Detection)[21] plugin and BiNGO (Biological Networks Gene Ontology tool)[22] plugin based on hypergeometric distribution were introduced for the Cytoscape platform to enable searches for dense clique-like structures within a network[23] and divided and annotated functional modules of the whole network. Degree cutoff and K-core (a subgraph in which all nodes have a degree at least k) of every module set to more than or equal to 2, and the *p*-value of hypergeometric distribution was less than 0.05.

Results

Screening for Differentially Expressed miRNAs

Total 40 significantly differentially expressed miRNAs between HCC and corresponding normal specimens were identified with FDR < 0.05 and |logFC| > 1.

Comparison of Differentially Expressed miRNAs Between Normal and HCC Samples

Figure 1A showed the expression difference of all miRNAs (left) and the differentially ex-

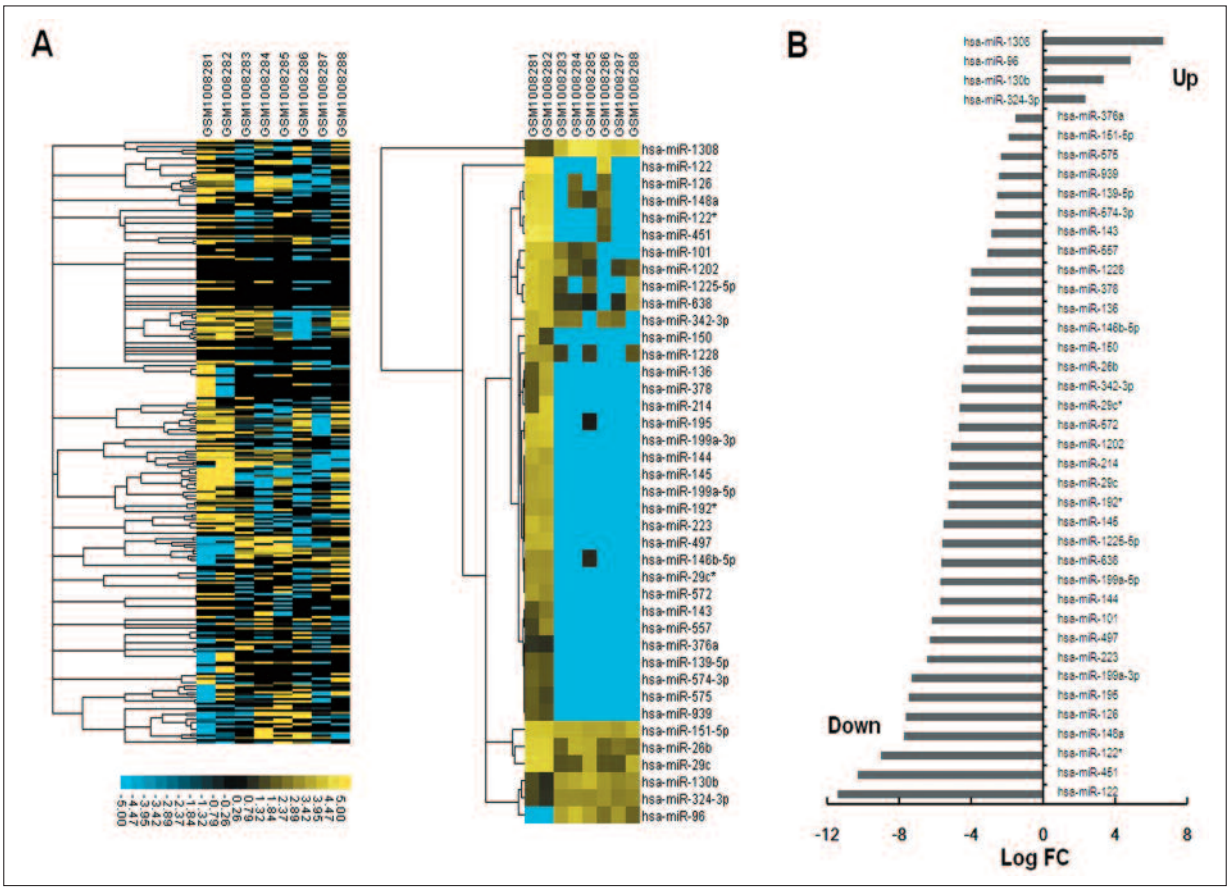

Figure 1. Clustering heat map of miRNA and sketch map of logFC. (A) The dendrogram of all miRNAs (left) and screening differentially expressed miRNAs (right). The yellow color represents up-regulation while the blue color indicates down-regulation. (B) The sketch map of logFC of differentially expressed miRNAs. The lower left and top right are down-regulated and up-regulated miRNAs, respectively.

pressed miRNAs (right) between normal and cancer samples. In addition, up-regulated hsa-miR-1308 and down-regulated hsa-miR-122 were the top two differentially expressed miRNAs with the highest |logFC| (Figure 1B).

Identification of miRNA-Target Genes

Combined with miRecords and miRTarBase databases, we searched reliable target genes of hsa-miR-1308 and hsa-miR-122. Target genes of hsa-miR-122, which were existed in both two databases, included 29 target genes with a high degree of confidence. However, no target genes of has-miR-1308 were available in these two databases.

Construction of Interaction Network

We mapped the target genes of hsa-miR-122 to STRING database, screened significant interactions and construct target genes interaction network. Figure 2 showed the interaction network of 629 pairs of mutual interactions. Among 29 target genes, ADAM17 (a disintegrin and metalloproteinase-17), NCAM1 (neural cell adhesion molecule 1) with high degree formed local networks, suggesting their important roles in development of HCC.

Modules Divided and Gene Ontology Enrichment Analysis

Based on the degree cutoff and K-core of every module set to more than or equal to 2 and *p*-value less than 0.05, four functional modules were obtained (Figure 3). As shown in Figure 3, EGLN3 in module A, ALDOA in module B, NCAM1 and ANK2 in module C, and AACS in module D were high confident target genes, respectively. Gene Ontology enriched functional annotation of genes in each module was shown in Table I, including signal transmission, regulation of macromolecule metabolic process.

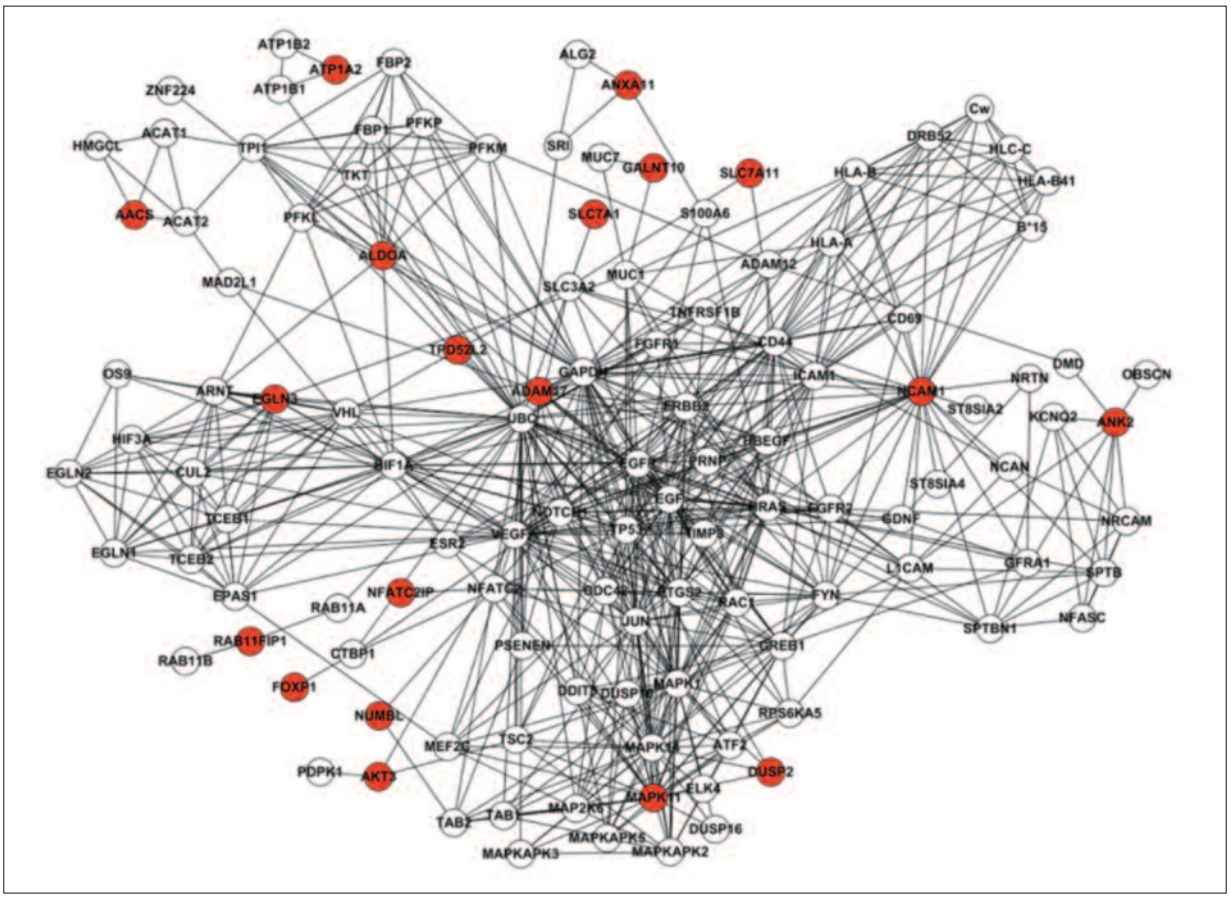

Figure 2. The interaction networks of high credible target genes. The red nodes are the high credible target genes, while the other nodes are the predictive interactive objects.

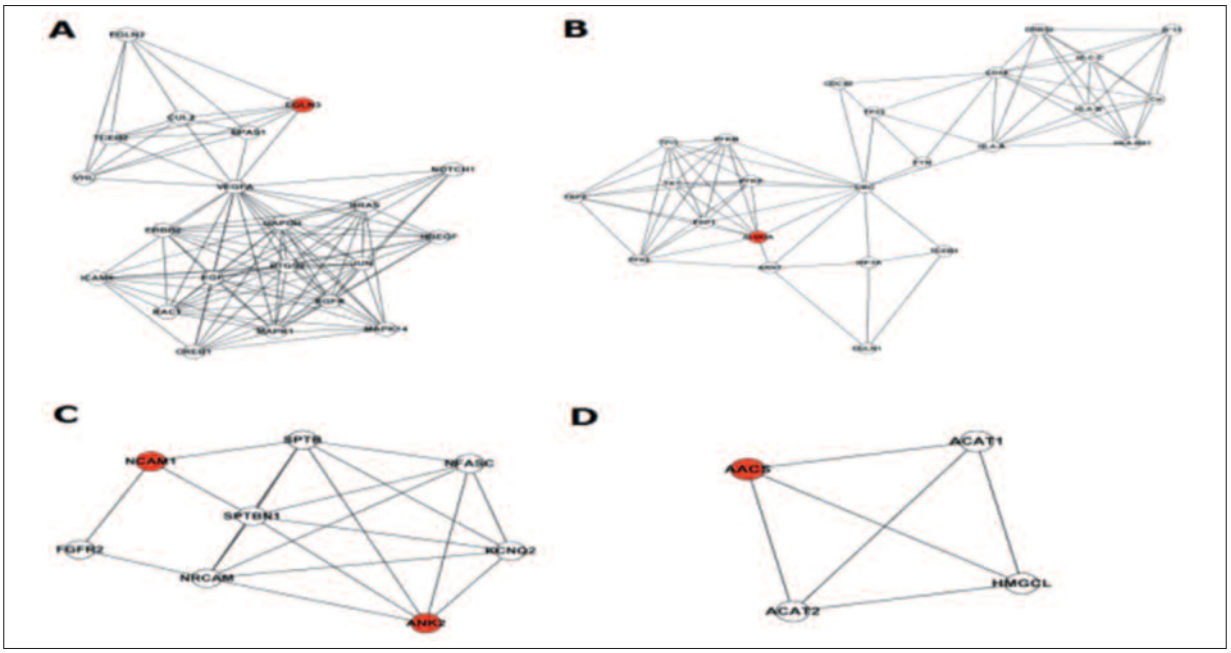

Figure 3. The functional modules of miRNA-target genes. The red nodes are the high credible target genes. Others are the predictive interactive objects.

Table I. The GO enrichment analysis of target genes in modules.

GO-ID	corr *p*-value	x	Description
a) Module 1			
60255	0.000095486	15	Regulation of macromolecule metabolic process
80090	0.0001601	15	Regulation of primary metabolic process
8152	0.018675	15	Metabolic process
48522	6.6669E-08	16	Positive regulation of cellular process
50896	0.000043178	16	Response to stimulus
31323	0.000052342	16	Regulation of cellular metabolic process
48518	5.6325E-08	17	Positive regulation of biological process
19222	0.000017416	17	Regulation of metabolic process
50794	0.00002298	20	Regulation of cellular process
50789	0.000049836	20	Regulation of biological process
65007	0.000115	20	Biological regulation
9987	0.0078364	20	Cellular process
b) Module 2			
6000	2.80E-14	6	Fructose metabolic process
44419	2.88E-06	6	Interspecies interaction between organisms
51704	3.92E-04	6	Multi-organism process
2376	1.07E-03	6	Immune system process
9056	1.46E-03	6	Catabolic process
6006	4.54E-10	7	Glucose metabolic process
19318	2.39E-09	7	Hexose metabolic process
5996	7.02E-09	7	Monosaccharide metabolic process
44262	3.38E-07	7	Cellular carbohydrate metabolic process
6066	8.16E-07	7	Alcohol metabolic process
5975	2.85E-06	7	Carbohydrate metabolic process
44281	1.30E-03	7	Small molecule metabolic process
42221	1.94E-03	7	Response to chemical stimulus
44237	2.97E-03	13	Cellular metabolic process
c) Module 3			
32535	0.0069906	3	Regulation of cellular component size
90066	0.0069906	3	Regulation of anatomical structure size
51128	0.020725	3	Regulation of cellular component organization
7155	0.02986	3	Cell adhesion
22610	0.02986	3	Biological adhesion
65008	0.033617	4	Regulation of biological quality
23052	0.017097	6	Signaling
d) Module 4			
44255	0.001654	3	Cellular lipid metabolic process
6629	0.0037065	3	Lipid metabolic process
44281	0.00076611	4	Small molecule metabolic process

Discussion

HCC is the most common primary liver cancer that accounts for 80% of all liver cancer cases. The annual number of new cases of HCC worldwide is over one million[24]. Recent advances have demonstrated that dysregulation of miRNA expression in tumor tissues of the liver and identified miRNA signatures that associated with tumor differentiation, diagnosis, staging, progression and response to therapy[10,11,25]. Therefore, comprehensive analysis of miRNAs expression using microarray technology will help us to better understand the relationship between aberrant miRNAs expression and cancers and find new biomarkers for cancers.

In our study, we identified 40 significantly differentially expressed miRNAs between normal and HCC cell lines. Hierarchical clustering analysis showed up-regulated hsa-miR-1308 and down-regulated hsa-miR-122 were the top two differentially expressed miRNAs. Furthermore,

we screened for miRNA target genes and found that 29 high-credible target genes of hsa-miR-122 were identified both in miRecords and miRTarBase databases.

MiRNA-122 was one of the first examples of a tissue-specific miRNA. The sequence of mature miR-122 is completely conserved between all species in which it has been detected, and no paralogs have been identified, suggesting that the entire sequence is important for function[26]. It is highly expressed in liver but absent from other tissues. MiRNA-122, modulates hepatic lipid metabolism[27], is often down-regulated in human HCC[28,29]. Loss of its expression correlates with loss of mitochondrial metabolic function and is detrimental to sustain critical liver function, thereby contributing to the morbidity and mortality of liver cancer patients[30]. Researchers have proved that both miRNA-122 and NDRG3 (N-myc down-regulated gene) are viable therapeutic targets for HBV-related HCC. For HCV infection, miRNA-122 binds directly to two sites in the 5' non-coding region of HCV genome and positively regulates the viral life cycle[31].

STRING software was utilized to predict the interacted objects of miRNA-122 target genes and construct interaction network. From the network, we could find that ADAM17 (a disintegrin and metalloproteinase-17) and NCAM1 (neural cell adhesion molecule 1) were the reliable target genes and in the hub of the network. ADAM17 is a member of the metalloproteinase super-family and involved in the cleavage of ectodomain of many transmembrane proteins. It is also overexpressed in a variety of human tumors, which is associated with tumor development and progression. The central role of ADAM-17 in cell regulation is rooted in its diverse array of substrates: cytokines, growth factors, and their receptors as well as adhesion molecules are activated or inactivated by their cleavage with ADAM-17[32]. ADAM17 is a critical downstream target of miR-122. Researchers have proved that miR-122, a tumor suppressor microRNA affecting HCC intrahepatic metastasis by angiogenesis suppression, exerts some of its action via regulation of ADAM17[33]. Recent study has demonstrated that ADAM17 mediates hypoxia-induced drug resistance in HCC cells through activation of EGFR pathway and indicates that ADAM17 is a potential therapeutic target for HCC treatment[34]. NCAM1, a known hepatic stem/progenitor cell marker, was experimentally demonstrated to be a direct target of miR-200c[35]. Soluble NCAM status was a significant independent factor predictive of long-term survival in patients with HCC, and high levels of soluble NCAM were significantly related to intrahepatic metastasis[36].

Based on Cytoscape software, we obtained 4 functional modules of target genes. EGLN3, ALDOA (aldolase A), NCAM1, ANK2 (ankyrin-B gene) and AACS (acetoacetyl-CoA synthetase) were five genes with high degree of confidence in these modules, respectively. EGLN3 has been described as the main actor in the response to chronic hypoxia and the regulation of Hif1□[37]. Researchers have demonstrated that KIF1B□, associated with HCC, can induce apoptosis by acting downstream of EGLN3 prolyhydroxylase, which may lead to inhibition of malignant transformation and progression[38,39]. Some studies have shown high levels of ALDOA expression in some neoplasias, such as lung adenocarcinomas and HCC[40]. ANK2 is mainly expressed in brain, striated muscle, kidney, thymus, and peripheral blood cells[41,42]. Researchers have demonstrated that ANK2 mutation can cause type 4 long-QT cardiac arrhythmia and sudden cardiac death[43]. AACS, an essential enzyme for the synthesis of fatty acid and cholesterol from ketone bodies, was possibly in response to the rise in the levels of acetyl-CoA[44].

Gene ontology analysis for these genes included macromolecule metabolic process, cellular component size, cellular lipid metabolic process and so on. EGFR (epidermal growth factor receptor) and ICAM (intercellular adhesion molecule) were two of the most active genes in test set. The EGFR signaling system is commonly activated in HCC, and is currently being evaluated as a therapeutic target in combination therapies[45]. Researchers have identified a central role for the EGFR ligand amphiregulin (AR) in the proliferation, survival and drug resistance of HCC cells[46]. Furthermore, EGFR is associated with sex bias occurrence of HCC in Poly7 molecular subclass[47]. ICAM-1 is considered closely related to occurrence, development, metastasis and invasion processes of HCC[48].

Recent studies have shown that miRNAs control different aspects of energy metabolism, including insulin production and signaling, glucose transport and metabolism, cholesterol and lipid homeostasis, cellular lipid metabolic process and amino acid biogenesis[49,50]. MiRNAs regulate cell metabolic processes either directly by targeting key molecules of metabolic pathways (transporters, enzymes, and kinases) or indirectly by

modulating the expression of important transcription factors[51]. Lipid metabolism is known to be an important process involved in hepatic steatosis[52]. Study has proved that the pathway category lipid metabolism was the most affected metabolic process in HCC[53].

The miRNAs therapy for HCC is becoming a popular subject in the cancer research. Moreover, there are many studies that report the association between miRNAs expression and HCC.

Conclusions

In this article, we identified the differentially expressed hsa-miR-122 by comparing HCC samples with controls. Based on subsequent analysis, we predict miR-122 play an important role in the development of HCC. MiR-122 as a single biomarker could be useful in the diagnosis of HCC. However, further experiments are needed to verify our results.

Acknowledgements

This work was supported by Natural Science Foundation Project of Shanghai (Grant no. 12ZR1437900).

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Waly Raphael S, Yangde Z, Yuxiang C. Hepatocellular carcinoma: focus on different aspects of management. ISRN Oncol 2012; 2012: 421673.
2) Yang JD, Roberts LR. Epidemiology and management of hepatocellular carcinoma. Infect Dis Clin North Am 2010; 24: 899-919, viii.
3) Huang XB, Li J, Zheng L, Zuo GH, Han KQ, Li HY, Liang P. Bioinformatics analysis reveals potential candidate drugs for HCC. Pathol Oncol Res 2013; 19: 251-258.
4) Roux J, Gonzalez-Porta M, Robinson-Rechavi M. Comparative analysis of human and mouse expression data illuminates tissue-specific evolutionary patterns of miRNAs. Nucleic Acids Res 2012; 40: 5890-5900.
5) Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res 2011; 39: 152-157.
6) Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell 2009; 136: 642-655.
7) Somel M, Guo S, Fu N, Yan Z, Hu HY, Xu Y, Yuan Y, Ning Z, Hu Y, Menzel C. MicroRNA, mRNA, and protein expression link development and aging in human and macaque brain. Genome Res 2010; 20: 1207-1218.
8) Somel M, Liu X, Tang L, Yan Z, Hu H, Guo S, Jiang X, Zhang X, Xu G, Xie G, Li N, Hu Y, Chen W, Paabo S, Khaitovich P. MicroRNA-driven developmental remodeling in the brain distinguishes humans from other primates. PLoS Biol 2011; 9: e1001214.
9) Borel F, Konstantinova P, Jansen PL. Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma. J Hepatol 2012; 56: 1371-1383.
10) Braconi C, Henry JC, Kogure T, Schmittgen T, Patel T. The role of microRNAs in human liver cancers. Semin Oncol 2011; 38: 752-763.
11) Huang S, He X. The role of microRNAs in liver cancer progression. Br J Cancer 2011; 104: 235-240.
12) Law PT, Wong N. Emerging roles of microRNA in the intracellular signaling networks of hepatocellular carcinoma. J Gastroenterol Hepatol 2011; 26: 437-449.
13) Furuta M, Kozaki K, Tanimoto K, Tanaka S, Arii S, Shimamura T, Niida A, Miyano S, Inazawa J. The tumor-suppressive miR-497-195 cluster targets multiple cell-cycle regulators in hepatocellular carcinoma. PLoS One 2013; 8: e60155.
14) Best CJ, Gillespie JW, Yi Y, Chandramouli GV, Perlmutter MA, Gathright Y, Erickson HS, Georgevich L, Tangrea MA, Duray PH, Gonzalez S, Velasco A, Linehan WM, Matusik RJ, Price DK, Figg WD, Emmert-Buck MR, Chuaqui RF. Molecular alterations in primary prostate cancer after androgen ablation therapy. Clin Cancer Res 2005; 11: 6823-6834.
15) Smyth GK, Yang YH, Speed T. Statistical issues in cDNA microarray data analysis. Methods Mol Biol 2003; 224: 111-136.
16) Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A 1998; 95: 14863-14868.
17) Xiao F, Zuo Z, Cai G, Kang S, Gao X, Li T. miRecords: an integrated resource for microRNA-target interactions. Nucleic Acids Res 2009; 37: 105-110.
18) Hsu SD, Lin FM, Wu WY, Liang C, Huang WC, Chan WL, Tsai WT, Chen GZ, Lee CJ, Chiu CM, Chien CH, Wu MC, Huang CY, Tsou AP, Huang HD. miRTarBase: a database curates experimentally validated microRNA-target interactions. Nucleic Acids Res 2011; 39: 163-169.
19) Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, Doerks T, Stark M, Muller J, Bork P, Jensen LJ, von Mering C. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res 2011; 39: 561-568.

20) Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics 2011; 27: 431-432.

21) Bandettini WP, Kellman P, Mancini C, Booker OJ, Vasu S, Leung SW, Wilson JR, Shanbhag SM, Chen MY, Arai AE. MultiContrast Delayed Enhancement (MCODE) improves detection of subendocardial myocardial infarction by late gadolinium enhancement cardiovascular magnetic resonance: a clinical validation study. J Cardiovasc Magn Reson 2012; 14: 83.

22) Maere S, Heymans K, Kuiper M. BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. Bioinformatics 2005; 21: 3448-3449.

23) Rivera CG, Vakil R, Bader JS. NeMo: Network Module identification in Cytoscape. BMC Bioinformatics 2010; 11(Suppl 1): S61.

24) Nordenstedt H, White DL, El-Serag HB. The changing pattern of epidemiology in hepatocellular carcinoma. Dig Liver Dis 2010; 42(Suppl 3): S206-214.

25) Negrini M, Gramantieri L, Sabbioni S, Croce CM. microRNA involvement in hepatocellular carcinoma. Anticancer Agents Med Chem 2011; 11: 500-521.

26) Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. Curr Biol 2002; 12: 735-739.

27) Lindow M, Kauppinen S. Discovering the first microRNA-targeted drug. J Cell Biol 2012; 199: 407-412.

28) Jopling C. Liver-specific microRNA-122: Biogenesis and function. RNA Biol 2012; 9: 137-142.

29) Kutay H, Bai S, Datta J, Motiwala T, Pogribny I, Frankel W, Jacob ST, Ghoshal K. Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. J Cell Biochem 2006; 99: 671-678.

30) Burchard J, Zhang C, Liu AM, Poon RT, Lee NP, Wong K-F, Sham PC, Lam BY, Ferguson MD, Tokiwa G. microRNA-122 as a regulator of mitochondrial metabolic gene network in hepatocellular carcinoma. Mol Syst Biol 2010; 6: 402.

31) Fan CG, Wang CM, Tian C, Wang Y, Li L, Sun WS, Li RF, Liu YG. miR-122 inhibits viral replication and cell proliferation in hepatitis B virus-related hepatocellular carcinoma and targets NDRG3. Oncol Rep 2011; 26: 1281-1286.

32) Gooz M. ADAM-17: the enzyme that does it all. Crit Rev Biochem Mol Biol 2010; 45: 146-169.

33) Tsai WC, Hsu PWC, Lai TC, Chau GY, Lin CW, Chen CM, Lin CD, Liao YL, Wang JL, Chau YP. MicroRNA©\122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. Hepatology 2009; 49: 1571-1582.

34) Wang X-J, Feng C-W, Li M. ADAM17 mediates hypoxia-induced drug resistance in hepatocellular carcinoma cells through activation of EGFR/PI3K/Akt pathway. Mol Cell Biochem 2013; 380: 57-66.

35) Oishi N, Kumar MR, Roessler S, Ji J, Forgues M, Budhu A, Zhao X, Andersen JB, Ye QH, Jia HL, Qin LX, Yamashita T, Woo HG, Kim YJ, Kaneko S, Tang ZY, Thorgeirsson SS, Wang XW. Transcriptomic profiling reveals hepatic stem-like gene signatures and interplay of miR-200c and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma. Hepatology 2012; 56: 1792-1803.

36) Tsuchiya A, Kamimura H, Tamura Y, Takamura M, Yamagiwa S, Suda T, Nomoto M, Aoyagi Y. Hepatocellular carcinoma with progenitor cell features distinguishable by the hepatic stem/progenitor cell marker NCAM. Cancer Lett 2011; 309: 95-103.

37) Ginouves A, Ilc K, Macias N, Pouyssegur J, Berra E. PHDs overactivation during chronic hypoxia "desensitizes" HIFalpha and protects cells from necrosis. Proc Natl Acad Sci U S A 2008; 105: 4745-4750.

38) Wang ZC, Gao Q, Shi JY, Yang LX, Zhou J, Wang XY, Shi YH, Ke AW, Shi GM, Ding ZB, Dai Z, Qiu SJ, Fan J. Genetic polymorphism of the kinesin-like protein KIF1B gene and the risk of hepatocellular carcinoma. PLoS One 2013; 8: e62571.

39) Zhang H, Zhai Y, Hu Z, Wu C, Qian J, Jia W, Ma F, Huang W, Yu L, Yue W. Genome-wide association study identifies 1p36. 22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. Nat Genet 2010; 42: 755-758.

40) Lessa RC, Campos AH, de Freitas CE, da Silva FR, Kowalski LP, Carvalho AL, Vettore AL. Identification of upregulated genes in oral squamous cell carcinomas. Head Neck 2013; 35: 1475-1481.

41) Otto E, Kunimoto M, McLaughlin T, Bennett V. Isolation and characterization of cDNAs encoding human brain ankyrins reveal a family of alternatively spliced genes. J Cell Biol 1991; 114: 241-253.

42) Leussis MP, Madison JM, Petryshen TL. Ankyrin 3: genetic association with bipolar disorder and relevance to disease pathophysiology. Biol Mood Anxiety Disord 2012; 2: 18.

43) Mohler PJ, Schott JJ, Gramolini AO, Dilly KW, Guatimosim S, duBell WH, Song LS, Haurogne K, Kyndt F, Ali ME, Rogers TB, Lederer WJ, Escande D, Le Marec H, Bennett V. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. Nature 2003; 421: 634-639.

44) Kirpich IA, Gobejishvili LN, Bon Homme M, Waigel S, Cave M, Arteel G, Barve SS, McClain CJ, Deaciuc IV. Integrated hepatic transcriptome and proteome analysis of mice with high-fat diet-induced nonalcoholic fatty liver disease. J Nutr Biochem 2011; 22: 38-45.

45) Lutasa MU, Salis F, Urtasun R, Garcia-Irigoyen O, Elizalde Ma, Uriarte I, Santamaria M, Feo F, Pascale RM, Prieto Js. Regulation of Amphiregulin gene expression by -catenin signaling in human Hepa-

tocellular carcinoma cells: a novel crosstalk between FGF19 and the EGFR system. PLoS One 2012; 7: e52711.

46) Urtasun R, Latasa MU, Demartis MI, Balzani S, Goni S, Garcia-Irigoyen O, Elizalde M, Azcona M, Pascale RM, Feo F, Bioulac-Sage P, Balabaud C, Muntane J, Prieto J, Berasain C, Avila MA. Connective tissue growth factor autocriny in human hepatocellular carcinoma: oncogenic role and regulation by epidermal growth factor receptor/yes-associated protein-mediated activation. Hepatology 2011; 54: 2149-2158.

47) Keng VW, Sia D, Sarver AL, Tschida BR, Fan D, Alsinet C, Sol¨¦ M, Lee WL, Kuka TP, Moriarity BS. Sex bias occurrence of hepatocellular carcinoma in Poly7 molecular subclass is associated with EGFR. Hepatology 2013; 57: 120-130.

48) Zhu XW, Gong JP. Expression and role of icam-1 in the occurrence and development of hepatocellular carcinoma. Asian Pac J Cancer Prev 2013; 14: 1579-1583.

49) Rottiers V, Naar AM. MicroRNAs in metabolism and metabolic disorders. Nat Rev Mol Cell Biol 2012; 13: 239-250.

50) Chen B, Li H, Zeng X, Yang P, Liu X, Zhao X, Liang S. Roles of microRNA on cancer cell metabolism. J Transl Med 2012; 10: 228.

51) Hatziapostolou M, Polytarchou C, Iliopoulos D. miRNAs link metabolic reprogramming to oncogenesis. Trends Endocrinol Metab 2013; 24: 361-373.

52) Wu JM, Skill NJ, Maluccio MA. Evidence of aberrant lipid metabolism in hepatitis C and hepatocellular carcinoma. HPB (Oxford) 2010; 12: 625-636.

53) Becker D, Sfakianakis I, Krupp M, Staib F, Gerhold-Ay A, Victor A, Binder H, Blettner M, Maass T, Thorgeirsson S, Galle PR, Teufel A. Genetic signatures shared in embryonic liver development and liver cancer define prognostically relevant subgroups in HCC. Mol Cancer 2012; 11: 55.