SPANDIDOS PUBLICATIONS

MOLECULAR MEDICINE REPORTS 15: 3681-3689, 2017

Bioinformatics analysis of gene expression alterations in microRNA-122 knockout mice with hepatocellular carcinoma

BOSHENG HE[1*], YING HE[2*], WEIXIANG SHI[1], SHENCHU GONG[1], XIAOHONG CHEN[3], JING XIAO[4], JINHUA GU[5], WENBIN DING[1] and YILANG WANG[6]

[1]Department of Radiology, The Second Affiliated Hospital of Nantong University, Nantong, Jiangsu 226001; [2]Department of Ultrasound, The Tumor Hospital of Nantong University, Nantong, Jiangsu 226361; [3]Department of Ultrasound, The Second Affiliated Hospital of Nantong University, Nantong, Jiangsu 226001; [4]Department of Epidemiology and Medical Statistics, School of Public Health, Nantong University, Nantong, Jiangsu 226019; [5]Department of Pathophysiology, Nantong University Medical School; [6]Department of Oncology, The Second Affiliated Hospital of Nantong University, Nantong, Jiangsu 226001, P.R. China

Received December 31, 2015; Accepted February 6, 2017

DOI: 10.3892/mmr.2017.6445

Abstract. Reduced microRNA (miR)-122 expression levels are frequently observed in hepatocellular carcinoma (HCC). The present study was conducted to investigate potential targets of miR-122 and determine the underlying regulatory mechanisms of miR-122 in HCC development. The public dataset GSE31731 was utilized, consisting of 8 miR-122 knockout (KO) mice (miR-122 KO) and 8 age-matched wild-type mice (WT group). Following data preprocessing, the differentially expressed genes (DEGs) were selected, followed by enrichment analysis. A protein-protein interaction (PPI) network was established, and a module network was further extracted. Combining the DEGs with microRNA targeting databases permitted the screening of the overlapping targets of miR-122. Furthermore, previously reported genes were screened out by literature mining. Transcription factors (TFs) of the targets were subsequently investigated. DEGs between miR-122 KO and WT groups were selected, including 713 upregulated and 395 downregulated genes. Of these, upregulated genes were enriched in cell cycle-associated processes [including nucleolar and spindle associated protein 1 (*NUSAP1*)], the cytokine-cytokine receptor interaction pathway [including C-X-C motif chemokine receptor 4 (*CXCR4*) and C-C motif chemokine receptor 2 (*CCR2*)], and the extracellular matrix-receptor interaction pathway [including integrin subunit alpha V (*ITGAV*)]. In addition, multiple overlapping targets were highlighted in the PPI network, including *NUSAP1, CXCR4, CCR2* and *ITGAV*. Notably, *CXCR4* and *CCR2* were linked in module C, enriched in the cytokine-cytokine receptor interaction pathway. Furthermore, upregulated sex determining region Y-box 4 (*SOX4*) was identified as a TF. The results of the present study may provide a theoretical basis for further studies on the mechanisms of miR-122 in the development of HCC.

Correspondence to: Dr Wenbin Ding, Department of Radiology, The Second Affiliated Hospital of Nantong University, 6 Hai Er Xiang Road, Nantong, Jiangsu 226001, P.R. China
E-mail: dingwenbinyh@hotmail.com

Dr Yilang Wang, Department of Oncology, The Second Affiliated Hospital of Nantong University, 6 Hai Er Xiang Road, Nantong, Jiangsu 226001, P.R. China
E-mail: oncowang@163.com

[*]Contributed equally

Key words: hepatocellular carcinoma, differential analysis, microRNA-122, target, bioinformatics, transcription factor

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the most frequent liver cancer globally (1). The majority of HCC cases occur in cirrhotic liver, and the primary risk factors are chronic hepatitis B virus or chronic hepatitis C virus (HCV) infection, which account for almost all HCC cases (2). The incidence of HCC varies between different geographical areas; however, it is increasing globally, particularly in Asia, with 6-11 per 100,000 people with the disease (3,4). A study of HCC epidemiology in Germany indicated that, despite the availability of various advanced chemotherapies and radiotherapies, including chemoembolization with drug-eluting beads, sorafenib and selective internal radiotherapy, the overall survival rate has not improved (5). Therefore, the development of more effective therapeutic methods, including molecular targeting therapy, is necessary.

Multiple studies have been conducted to investigate the molecular mechanisms underlying HCC pathogenesis and numerous gene markers have been identified in HCC, including alpha-fetoprotein, glypican-3 (a serum and histochemical marker) and transforming growth factor-β (6-8). As small, non-coding RNAs, microRNAs (miRNAs) are important

regulators of cellular function and physiology (9). Controlling miRNA expression is essential for the maintenance of the steady state of cellular machinery (10). Various microRNAs (miRNAs) have been proposed as novel biomarkers of HCC prognosis, including the chromosome 19 miRNA cluster, which is overexpressed in HCC (11). HCV-induced alteration of miRNA expression regulates inflammation, leading to liver fibrosis. In addition, miRNA (miR)-449a has been reported to serve as an inhibitor in HCV patients, acting via the downregulation of chitinase-3-like protein 1 expression, which is an inflammatory marker for chronic liver diseases with fibrosis (12). Dysregulation of other miRNAs has been detected in HCC, including upregulated miR-23a, -146a and -181a, and downregulated miR-17, -338-3p and -378 (13). Notably, miR-122 is involved in HCC pathogenesis. It is commonly downregulated in HCC and the loss of miR-122 contributes to hepatocarcinogenesis in mice (14). miR-122 has additionally been reported to induce apoptosis in human HCC cell lines via targeting the anti-apoptosis gene B-cell lymphoma-2-like 2 (15). Furthermore, miR-122 inhibits cell proliferation in HCC by targeting the Wnt/β-catenin signaling pathway (16). To further understand the modulation of miR-122 in HCC development, previous studies have investigated the consequences of miR-122 deletion. Hsu *et al* (17) revealed that deletion of mouse mir-122 resulted in hepatocarcinogenesis and HCC-like tumor development. Although increased expression of multiple targets of miR-122 has been detected in miR-122 knockout (KO) mice, including aldolase, fructose bisphosphate A (*ALDOA*), solute carrier family 7 member 1, citrate synthase and cyclin G1, the functions and pathways of the targets, and the potential associations between them at the protein level, remain to be elucidated.

In the present study, the expression profile dataset generated by Hsu *et al* (17), GSE31731, was reanalyzed and differentially expressed genes (DEGs) were identified between miR-122 KO mice and age-matched wild-type (WT) mice. Enrichment analysis of the identified DEGs was subsequently conducted, followed by protein-protein interaction (PPI) and module analysis. Furthermore, targets of miR-122 of these DEGs were selected by combining the results with relevant databases and literature mining, followed by transcription factor (TF) analysis. By means of these comprehensive bioinformatics analyses, the present study aimed to further elucidate the involvement of miR-122 in HCC, and identify potential regulators among its targets.

Materials and methods

Data resources. Gene expression of GSE31731 of HCCs, which was deposited by Hsu *et al* (17) in the public Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo) database, was utilized in the present study. The miR-122 KO mice (liver tumor samples) and age-matched WT mice (healthy liver samples) were contained in this dataset. There were 8 biological replicates. Two-channel microarray experiments were conducted for generation of the dataset, based on the platform GPL13912 (accession no. Agilent-028005 SurePrint G3 Mouse GE 8x60 K Microarray; Agilent Technologies, Inc., Santa Clara, CA, USA).

Data pretreatment and differential expression analysis. Raw data were preprocessed using R package in Bioconductor (version 3.4; www.bioconductor.org/packages/3.0/bioc/). Following background correction and normalization, the expression value was converted from the probe level to the gene level. Subsequently, DEGs between liver tumor samples and healthy liver samples were screened out, based on the Student's *t*-test in Linear Models for Microarray Analysis package (version 3.30.3; www.bioconductor.org/packages/release/bioc/html/limma.html) (18). The Benjamini-Hochberg method (19) was used to adjust the P-value. The selection criteria for significant DEGs were a false discovery rate <0.01 and a $\log_2$ fold change >1.5.

Enrichment analysis for DEGs. The Database for Annotation, Visualization and Integrated Discovery (DAVID; david.ncifcrf.gov/home.jsp) is a common tool for gene function and pathway annotation (20-22). To examine biological functions and pathways of the identified DEGs, Gene Ontology (GO; www.geneontology.org/) (23) and Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg/pathway.html) (24) pathway enrichment analyses were performed using DAVID (version 6.8). Cut-off values for significant GO and KEGG pathway terms were P<0.05 and enriched gene number ≥2.

PPI network construction. To predict potential interactions between DEGs at the protein level, the DEGs were entered into the Search Tool for the Retrieval of Interacting Genes (string-db.org/) database (25). The parameter of interplayed PPIs was set as 0.7, and a prerequisite for the network construction was that all the PPI nodes were DEGs. Finally, the PPI network was visualized by Cytoscape (version 3.4.0; cytoscape.org/) software (26).

Furthermore, module analysis was performed for the PPI network using ClusterONE (version 1.0; www.paccanarolab.org/clusterone/) (27), followed by KEGG pathway enrichment analysis. The threshold for significant module selection was $P<1.0x10^{-6}$.

Analysis of miR-122 targets. Initially, targets of miR-122 in mouse were downloaded from three databases: miRecords (c1.accurascience.com/miRecords/) (28), TargetScan (www.targetscan.org/) (29) and microrna.org (www.microrna.org) (30), and only genes that appeared in at least two databases were deemed to be targets of miR-122. These predicted targets were compared with the DEGs, and the overlapping genes were screened out. Following this, TFs of the overlapped targets were predicted by the iRegulon plugin of Cytoscape (iregulon.aertslab.org), which integrates a set of TF databases including Transfac, Jaspar, Encode, Swissregulon and Homer to detect enriched TF motifs and their optimal sets of direct targets (31). Normalized Enrichment Score (NES) was the measurement index for TFs of the targets and the threshold used was NES >3. Furthermore, the Agilent Literature Search plugin (Agilent Technologies, Inc.) (32), which is complementary for protein interaction data, was used to analyze the literature mining association network. In the present study, the search terms were set as 'targets of miR-122', context 'liver cancer' and

Table I. Functions altered by differentially expressed genes.

Category	Term	Count	P-value
Upregulated			
BP	GO:0007049~cell cycle	57	$4.96x10^{-11}$
	GO:0000279~M phase	34	$1.64x10^{-09}$
	GO:0000278~mitotic cell cycle	31	$2.64x10^{-09}$
	GO:0007067~mitosis	27	$3.21x10^{-09}$
	GO:0000280~nuclear division	27	$3.21x10^{-09}$
CC	GO:0005576~extracellular region	133	$9.92x10^{-18}$
	GO:0044421~extracellular region part	74	$2.00x10^{-13}$
	GO:0005578~proteinaceous extracellular matrix	35	$7.33x10^{-09}$
	GO:0031012~extracellular matrix	35	$2.01x10^{-08}$
	GO:0044420~extracellular matrix part	18	$4.96x10^{-08}$
MF	GO:0005509~calcium ion binding	60	$1.62x10^{-07}$
	GO:0008009~chemokine activity	10	$4.56x10^{-06}$
	GO:0008201~heparin binding	14	$5.26x10^{-06}$
	GO:0042379~chemokine receptor binding	10	$5.74x10^{-06}$
	GO:0001871~pattern binding	17	$9.14x10^{-06}$
Downregulated			
BP	GO:0055114~oxidation-reduction	64	$9.82x10^{-28}$
	GO:0006631~fatty acid metabolic process	24	$4.36x10^{-13}$
	GO:0008202~steroid metabolic process	21	$1.75x10^{-11}$
	GO:0006694~steroid biosynthetic process	15	$3.73x10^{-11}$
	GO:0006956~complement activation	10	$1.37x10^{-08}$
CC	GO:0005777~peroxisome	22	$4.28x10^{-15}$
	GO:0042579~microbody	22	$4.28x10^{-15}$
	GO:0005792~microsome	26	$5.15x10^{-14}$
	GO:0042598~vesicular fraction	26	$1.13x10^{-13}$
	GO:0005739~mitochondrion	66	$7.86x10^{-11}$
MF	GO:0009055~electron carrier activity	34	$4.36x10^{-21}$
	GO:0020037~heme binding	23	$9.42x10^{-14}$
	GO:0046906~tetrapyrrole binding	23	$2.57x10^{-13}$
	GO:0005506~iron ion binding	33	$3.02x10^{-13}$

The top 5 functions are presented for each category, ranked by the enrichment significance. Up, upregulated differentially expressed genes; down, downregulated differentially expressed genes; BP, biological process; CC, cellular component; MF, molecular function; GO, gene ontology; count, gene numbers enriched in a specific gene ontology term.

species 'Mus', to select the reported HCC-associated literature involving miR-122.

Results

DEGs between miR-122 KO and WT groups. Using predefined criteria, DEGs between miR-122 KO and WT groups were screened out, including 713 upregulated and 395 downregulated genes.

Altered functions and pathways of DEGs. Based on GO and KEGG enrichment analyses, upregulated DEGs were identified to be significantly enriched in cell cycle associated biological processes (BPs), including the cell cycle, M phase and mitotic cell cycle [for example nucleolar and spindle associated protein 1 (*NUSAP1*); Table I], the cytokine-cytokine receptor interaction pathway [for example C-X-C motif chemokine receptor 4 (*CXCR4*) and C-C motif chemokine receptor 2 (*CCR2*); Table II], and various cancer-associated pathways, including small cell lung cancer and pathways in cancer [for example integrin subunit alpha V (*ITGAV*); Table II]. The downregulated DEGs were associated with oxidation-reduction (Table I) and metabolism-associated pathways, including drug metabolism, linoleic acid metabolism and retinol metabolism (Table II).

PPI network and functional module analysis. A PPI network was established, involving 549 nodes and 2,243 interplayed protein interactions (Fig. 1). Three sub-networks [modules A (Fig. 2A), B (Fig. 2B) and C (Fig. 2C)] were extracted from the PPI network. Enrichment analysis revealed that the majority of the genes in module A were upregulated and enriched in DNA replication and cell cycle-associated pathways, whereas the majority of genes in module B were downregulated and enriched in metabolism of xenobiotics by cytochrome P450 pathway (Fig. 3). In module C, the majority of the genes were upregulated and involved with the chemokine signaling

Table II. Pathways altered by differentially expressed genes.

Term	Count	Genes	P-value
Upregulated			
mmu04060:Cytokine-cytokine receptor interaction	27	CCL2, CXCL5, CXCR4, CXCL14, CCR2	4.82×10^{-06}
mmu04512:ECM-receptor interaction	15	COL3A1, LAMA2, ITGAV, COL1A2, LAMC1	4.87×10^{-06}
mmu04510:Focal adhesion	22	COL3A1, LAMA2, ITGAV, LAMC1	4.43×10^{-05}
mmu00480:Glutathione metabolism	10	GPX2, GSTA1, GSTA2, GSTM3, G6PDX	2.05×10^{-04}
mmu04110:Cell cycle	14	CCNB2, KMYT1, BUB1B, ESPL1, CDC20	0.001940583
mmu05222:Small cell lung cancer	11	LAMA2, COL4A1, ITGAV, LAMC2, LAMC1	0.002161153
mmu04810:Regulation of actin cytoskeleton	19	ITGAX, ITGAV, PDGFRB, PAK1, DIAP3	0.002891419
mmu04062:Chemokine signaling pathway	16	CCL2, CXCR4, CXCL16, CCR2, CX3CR1	0.006803694
mmu05200:Pathways in cancer	23	COL4A1, LAMA2, ITGAV, LAMC2, LAMC1	0.011257034
mmu00590:Arachidonic acid metabolism	9	GPX2, CBR1, CYP4F18, GPX3, GGT1 *et al*	0.018763259
Downregulated			
mmu00982:Drug metabolism	18	CYP2C37, CYP3A16, CYP2C54, CYP2C44, ADH4	1.83×10^{-12}
mmu00980:Metabolism of xenobiotics by cytochrome P450	17	CYP2C37, CYP3A16, CYP2C54, CYP2C44, CYP2C68	2.81×10^{-12}
mmu00591:Linoleic acid metabolism	14	CYP2J5, CYP2C37, CYP3A16, CYP2C54, CYP2C44	4.16×10^{-11}
mmu00830:Retinol metabolism	16	CYP2C37, CYP3A16, CYP2C54, CYP2C44, CYP2C68	6.09×10^{-11}
mmu03320:PPAR signaling pathway	16	ACOX1, ACSL1, CYP4A12A, HMGCS2, SCP2	5.86×10^{-10}
mmu00590:Arachidonic acid metabolism	15	CYP2J5, CYP2C37, CYP2C54, CYP2C44, CYP2J8	1.14×10^{-08}
mmu00120:Primary bile acid biosynthesis	8	CYP7B1, HSD3B7, CYP7A1, CYP8B1, SCP2	2.81×10^{-08}
mmu00071:Fatty acid metabolism	9	CYP4A12B, GCDH, ACOX1, ACSL1, ADH4	1.21×10^{-05}
mmu00140:Steroid hormone biosynthesis	9	CYP7B1, CYP3A16, HSD3B6, HSD17B2, CYP7A1	1.21×10^{-05}
mmu04610:Complement and coagulation cascades	11	MBL1, C8A, MBL2, C8B, CD55	1.40×10^{-05}

The top 10 pathways are presented, ranked by the enrichment significance. Up, upregulated differentially expressed genes; down, downregulated differentially expressed genes; count, gene numbers enriched in a specific pathway term.

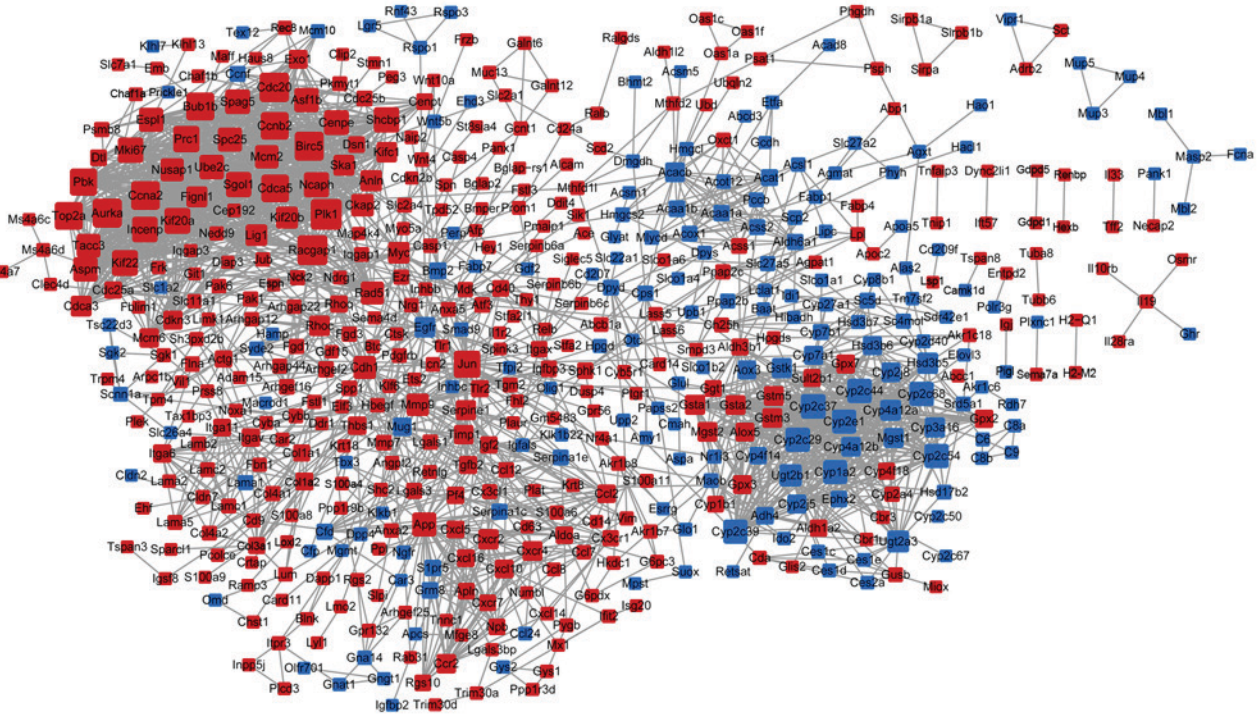

Figure 1. Protein-protein interaction network of differentially expressed genes. Red represents upregulated genes and blue represents downregulated genes. Lines between two genes denote interactions between them.

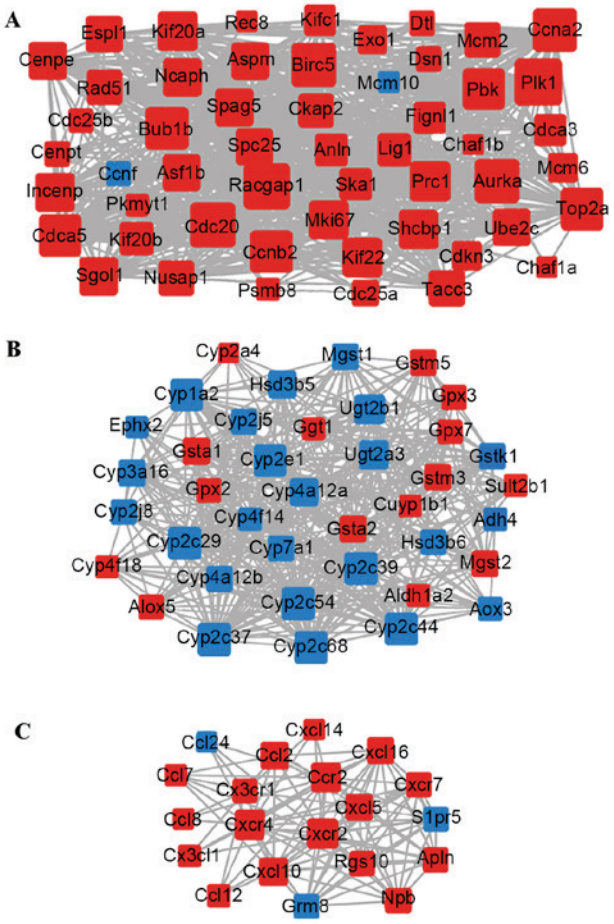

Figure 2. Module network of the protein-protein interaction network. (A) Module A, (B) module B and (C) module C networks. Red represents upregulated genes and blue represents downregulated genes. Lines between two genes denote interactions between them.

pathway and the cytokine-cytokine receptor interaction pathway (Fig. 3).

Targets of miR-122. Integrating the information from miRNA databases with identified DEGs, a total of 76 overlapping genes were selected as the targets of miR-122. Enrichment analysis indicated that these genes were significantly involved in pathways in cancer (for example *ITGAV*; Table II), regulation of actin cytoskeleton (for example *ITGAV*; Table II) and cytokine-cytokine receptor interaction (for example *CXCR4* and *CCR2*; Table II).

Notably, 39 genes of the 76 overlapping targets were additionally the predominant nodes with high degree in the PPI network, including upregulated *NUSAP1* (degree=30), *CXCR4* (degree=21), *CCR2* (degree=20), *ITGAV* (degree=17) and *ALDOA* (degree=14); and the downregulated acyl-CoA synthetase short-chain family member 2 (degree=10). *NUSAP1* was also highlighted in module A, whereas *CXCR4* and *CCR2* were prominent in module C.

In total, 12 TFs targeting 62 overlapping genes were predicted, including sex determining region Y-box 4 (*SOX4*), heterogeneous nuclear ribonucleoprotein H3, NK2 homeobox 1, inhibitor of growth family member 4, early B-cell factor 1, sex determining region Y-box 15, nuclear receptor subfamily 3 group C member 1, zinc finger protein 263, IKAROS family zinc finger 2, paired like homeodomain 3, eukaryotic translation initiation factor 5A2 and chromobox 7. The TF-target regulatory network is presented in Fig. 4. Notably, of the TFs, SOX4 was additionally an upregulated DEG.

According to literature mining, a total of 47 genes of the 76 overlapping targets were reported to be associated with HCC, including *ITGAV* and *CXCR4*. Furthermore, significantly altered expression of these genes was detected following miR-122 KO, which suggested the involvement of miR-122 in HCC development.

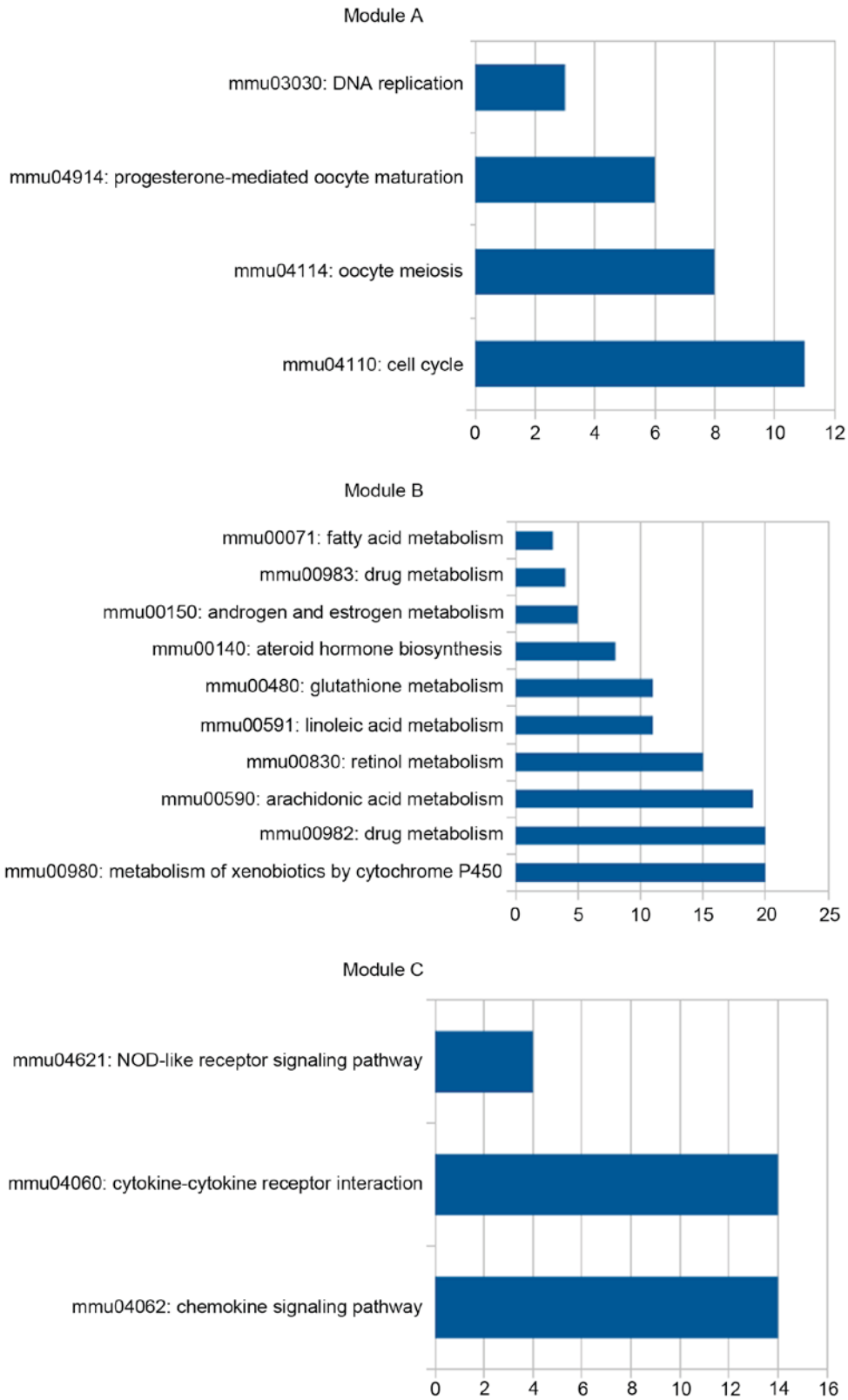

Figure 3. Functional enrichment analysis of the genes in each module network.

Discussion

miR-122 deficiency results in chronic steatohepatitis and spontaneous HCC (14). By re-analyzing the dataset GSE31731, numerous DEGs were identified between miR-122 KO and WT groups. Of these, upregulated genes were significantly enriched in cell cycle-associated processes (for example *NUSAP1*), cytokine-cytokine receptor interaction pathways (for example *CXCR4* and *CCR2*), and extracellular matrix (ECM) -receptor interactions (for example *ITGAV*). Various overlapping targets were highlighted in the PPI network, including *NUSAP1*, *CXCR4*, *CCR2* and *ITGAV*. Notably, *NUSAP1* was

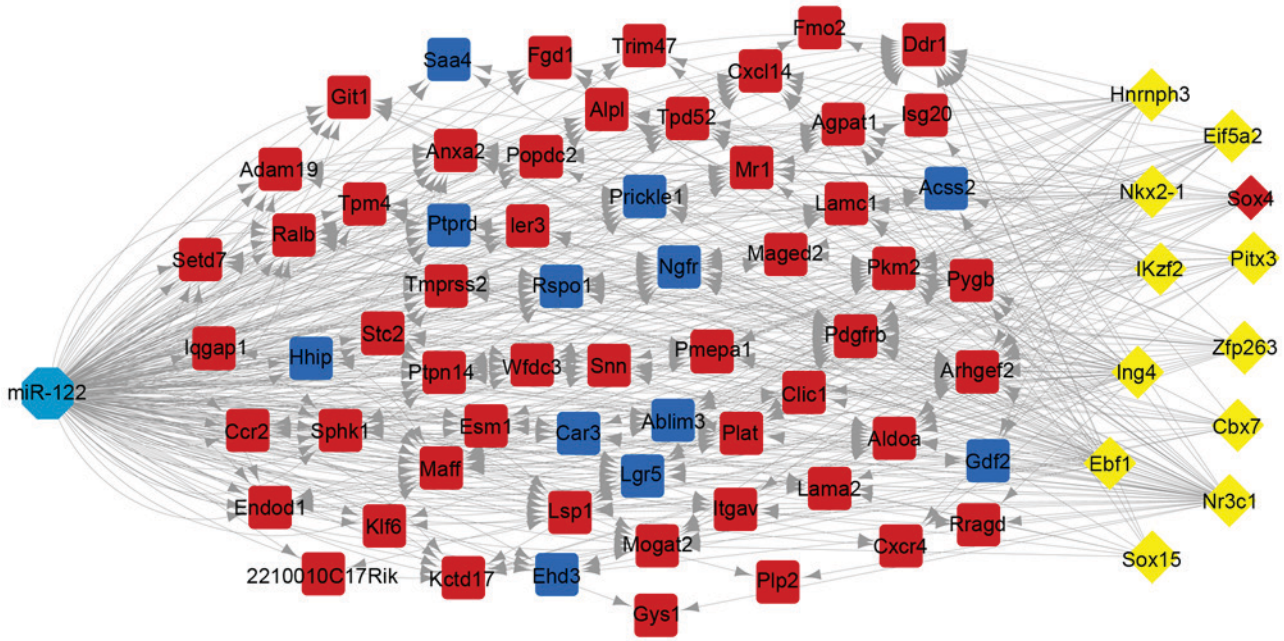

Figure 4. Targets of microRNA-122 and TFs of the targets. Squares represent targets (red, upregulated; blue, downregulated), diamonds represent TFs (red, upregulated; yellow, expression without significant difference). TF, transcription factor.

predominant in module A, which was associated with the cell cycle-associated pathway, whereas *CXCR4* and *CCR2* were linked in module C, enriched in the cytokine-cytokine receptor interaction pathway. Furthermore, upregulated *SOX4* was identified as a TF.

Restoration of miR-122 suppressed HCC tumor cell growth, and the antitumor activity was closely associated with cell cycle arrest (33). The protein encoded by *NUSAP1* is a nucleolar-spindle-associated protein that acts as a positive regulator of mitosis (34). It has been identified as a cell cycle progression gene in numerous cancer types, including prostate and lung cancer (35,36). In aggressive HCC, expression of *NUSAP1* is affected by other cell cycle-associated genes, including *L2DTL* (37). In the present study, upregulated *NUSAP1* in the miR-122 KO group was significantly enriched in cell cycle-associated BPs, and additionally served as a node in module A of the PPI network, as well as one of the overlapped targets of miR-122. However, *NUSAP1* has not been previously reported to be directly involved in HCC based on the literature mining results, suggesting that this gene may be a novel target of miR-122 involved in cell cycle-associated processes in HCC progression.

Dysregulation of the cytokine-cytokine receptor interaction pathway has been detected in HCC development (38,39). Activation of various chemokines in this pathway is involved in the development of numerous cancers, including HCC (40). As a chemokine receptor, the function of *CXCR4* has been extensively investigated. Increased expression of *CXCR4* has previously been reported to have a close association with the progression of HCC (41). In addition, *CXCR4* inhibition results in antitumor effects, including the inhibition of tumor growth and the improvement of survival in mice with HCC (42). Elevated expression of *CXCR4* in the miR-122 deficiency group, combined with the target information in miRNA databases, indicated that *CXCR4* may be a potential target of miR-122 in HCC. *CCR2* is the chemokine receptor of C-C motif chemokine ligand 2 (*CCL2*). Increased expression of *CCR2* has previously been observed in liver leukocytes from patients with HCC (43). *CCL2* is also involved in the cytokine-cytokine receptor interaction pathway and its expression is associated with HCC progression (38). Notably, *CCL2* has been identified as a target of miR-122, and restoration of miR-122 results in the suppression of *CCL2* in HCC (44). The results of the present study indicated that the DEG *CCR2* was upregulated in the miR-122 KO group and significantly enriched in the cytokine-cytokine receptor interaction pathway. Notably, it was additionally identified as an overlapping target based on the miRNA targeting database, and was linked to *CXCR4* in module C of the PPI network. These data collectively suggested that *CCR2* may be a target of miR-122, and co-regulate the cytokine-cytokine receptor interaction pathway with *CXCR4* in HCC development.

ECM-receptor interaction is a common pathway that is disturbed by altered gene expressions in various cancers (45,46). The gene *ITGAV* encodes a protein that belongs to the integrin superfamily. It has been reported to be involved in the ECM-receptor interaction pathway. It is a part of the ECM system (47), suggesting its involvement in the ECM-receptor interaction pathway. In other cancer types, including gastric cancer, *ITGAV* is enriched in this pathway (48). Notably, overexpressed *ITGAV* is induced by the gene forkhead box Q1 (*FOXQ1*), a member of the forkhead TF family that influences HCC metastasis (49), suggesting the potential involvement of this gene in HCC. Upregulated *ITGAV* in the miR-122 KO group, combined with enrichment analysis and the overlapped prediction target, indicated that this gene may be a target of miR-122 that modulates the ECM-receptor interaction pathway in HCC progression.

The intron-lacking gene *SOX4* contributes to hepatocarcinogenesis and its overexpression may be a useful prognostic

marker for survival after surgical resection (50). It has been demonstrated *in vitro* that overexpressed *SOX4* is involved in p53-mediated apoptosis in HCC (50). In addition, *SOX4* overexpression has previously been reported to control the metastasis of HCC (51). Thus, *SOX4* has been identified as a marker gene for HCC (52). Furthermore, *SOX4* serves as a TF that regulates cell differentiation. In HCC, various targets have been experimentally validated using chromatin immunoprecipitation and small interfering RNA assays, including aldo-keto reductase family 1 member B10, coiled-coil domain containing 97, dickkopf Wnt signaling pathway inhibitor 1, *FOXQ1* and microtubule associated protein 4 (51). miR-191 inhibition has previously been reported to result in the upregulation of *SOX4*, and increased *SOX4* expression promotes cell apoptosis and suppresses tumorigenesis of HCC (53). The present study indicated that the TF *SOX4* may additionally be a target of miR-122.

Despite these comprehensive bioinformatics analyses, the present study has limitations. The data was downloaded from the GEO database, and the sample size was relatively small. In addition, experimental validation of the associations between miR-122 and the predicted targets was lacking, and will be addressed in follow-up studies. Furthermore, the target expression levels following miR-122 restoration were not investigated, although this would have further confirmed the targeting associations. However, the present study has significant value as it provides novel insights into the consequences of miR-122 KO in HCC progression.

In conclusion, various crucial targets of miR-122 in HCC progression were identified, including *NUSAP1*, *CXCR4*, *CCR2* and *ITGAV*. Cell cycle-associated processes, the cytokine-cytokine receptor interaction pathway, which may be co-regulated by *CXCR4* and *CCR2*, and the ECM-receptor interaction pathway were altered by these targets. In addition, the target *SOX4* may be a TF. The results of the present study may provide a theoretical basis for further studies on the mechanisms of miR-122 in the development of HCC.

Acknowledgements

The present study was supported by the Social Development Fund of Nantong (grant no. HS2014063), the 12th Talent Summit of Top Six Industries in Jiangsu Province (grant no. WSW080) and the Project of Enhancing Medicine with Science and Education under the 13th Five-Year Plan of Nantong [grant no. Nantong MED Sci & Edu (2016) 23].

References

1. Yang J, Cai X, Lu W, Hu C, Xu X, Yu Q and Cao P: Evodiamine inhibits STAT3 signaling by inducing phosphatase shatterproof 1 in hepatocellular carcinoma cells. Cancer Lett 328: 243-251, 2013.
2. Borel F, Konstantinova P and Jansen PL: Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma. J Hepatol 56: 1371-1383, 2012.
3. Al-Mahtab M, Uddin H and Fazle Akbar SM: Epidemiology and Risk Factors of Hepatocellular Carcinoma in Asia. J Gastroenterology and Hepatology Res 3: 2014.
4. Paranaguá-Vezozzo DC, Ono SK, Alvarado-Mora MV, Farias AQ, Cunha-Silva M, França JI, Alves VA, Sherman M and Carrilho FJ: Epidemiology of HCC in Brazil: Incidence and risk factors in a ten-year cohort. Ann Hepatol 13: 386-393, 2014.
5. Weinmann A, Koch S, Niederle IM, Schulze-Bergkamen H, König J, Hoppe-Lotichius M, Hansen T, Pitton MB, Düber C, Otto G, *et al*: Trends in epidemiology, treatment, and survival of hepatocellular carcinoma patients between 1998 and 2009: An analysis of 1066 cases of a German HCC registry. J Clin Gastroenterol 48: 279-289, 2014.
6. Soresi M, Magliarisi C, Campagna P, Leto G, Bonfissuto G, Riili A, Carroccio A, Sesti R, Tripi S and Montalto G: Usefulness of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. Anticancer Res 23: 1747-1753, 2003.
7. Capurro M, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E and Filmus J: Glypican-3: A novel serum and histochemical marker for hepatocellular carcinoma. Gastroenterology 125: 89-97, 2003.
8. Murata M, Matsuzaki K, Yoshida K, Sekimoto G, Tahashi Y, Mori S, Uemura Y, Sakaida N, Fujisawa J, Seki T, *et al*: Hepatitis B virus X protein shifts human hepatic transforming growth factor (TGF)-beta signaling from tumor suppression to oncogenesis in early chronic hepatitis B. Hepatology 49: 1203-1217, 2009.
9. Beta M, Venkatesan N, Vasudevan M, Vetrivel U, Khetan V and Krishnakumar S: Identification and insilico analysis of retinoblastoma serum microRNA profile and gene targets towards prediction of novel serum biomarkers. Bioinform Biol Insights 7: 21-34, 2013.
10. Kosaka N, Iguchi H and Ochiya T: Circulating microRNA in body fluid: A new potential biomarker for cancer diagnosis and prognosis. Cancer Sci 101: 2087-2092, 2010.
11. Augello C, Vaira V, Caruso L, Destro A, Maggioni M, Park YN, Montorsi M, Santambrogio R, Roncalli M and Bosari S: MicroRNA profiling of hepatocarcinogenesis identifies C19MC cluster as a novel prognostic biomarker in hepatocellular carcinoma. Liver Int 32: 772-782, 2012.
12. Sarma NJ, Tiriveedhi V, Subramanian V, Shenoy S, Crippin JS, Chapman WC and Mohanakumar T: Hepatitis C virus mediated changes in miRNA-449a modulates inflammatory biomarker YKL40 through components of the NOTCH signaling pathway. PLoS One 7: e50826, 2012.
13. Zhang ZZ, Liu X, Wang DQ, Teng MK, Niu LW, Huang AL and Liang Z: Hepatitis B virus and hepatocellular carcinoma at the miRNA level. World J Gastroenterol 17: 3353-3358, 2011.
14. Hsu SH, Wang B, Kutay H, Bid H, Shreve J, Zhang X, Costinean S, Bratasz A, Houghton P and Ghoshal K: Hepatic loss of miR-122 predisposes mice to hepatobiliary cyst and hepatocellular carcinoma upon diethylnitrosamine exposure. Am J Pathol 183: 1719-1730, 2013.
15. Lin CJ, Gong HY, Tseng HC, Wang WL and Wu JL: miR-122 targets an anti-apoptotic gene, Bcl-w, in human hepatocellular carcinoma cell lines. Biochem Biophys Res Commun 375: 315-320, 2008.
16. Xu J, Zhu X, Wu L, Yang R, Yang Z, Wang Q and Wu F: MicroRNA-122 suppresses cell proliferation and induces cell apoptosis in hepatocellular carcinoma by directly targeting Wnt/β-catenin pathway. Liver Int 32: 752-760, 2012.
17. Hsu SH, Wang B, Kota J, Yu J, Costinean S, Kutay H, Yu L, Bai S, La Perle K, Chivukula RR, *et al*: Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. J Clin Invest 122: 2871-2883, 2012.
18. Smyth GK: Limma: Linear models for microarray data. In: Bioinformatics and computational biology solutions using R and Bioconductor. Springer, pp397-420, 2005.
19. Haynes W: Benjamini-Hochberg Method. In: Encyclopedia of Systems Biology. Springer, pp78-78, 2013.
20. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC and Lempicki RA: DAVID: Database for annotation, visualization and integrated discovery. Genome Biol 4: P3, 2003.
21. Huang da W, Sherman BT and Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4: 44-57, 2009.
22. Huang da W, Sherman BT and Lempicki RA: Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37: 1-13, 2009.
23. Gene Ontology Consortium; Blake JA, Dolan M, Drabkin H, Hill DP, Li N, Sitnikov D, Bridges S, Burgess S, Buza T, McCarthy F, *et al*: Gene Ontology annotations and resources. Nucleic Acids Res 41: D530-D535, 2013.
24. Kanehisa M, Goto S, Sato Y, Furumichi M and Tanabe M: KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Res 40: D109-D114, 2012.

25. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, *et al*: STRING v10: Protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 43: D447-D452, 2015.
26. Smoot ME, Ono K, Ruscheinski J, Wang PL and Ideker T: Cytoscape 2.8: New features for data integration and network visualization. Bioinformatics 27: 431-432, 2011.
27. Wan FC, Cui YP, Wu JT, Wang JM, -Z Liu Q and Gao ZL: The PPI network and cluster ONE analysis to explain the mechanism of bladder cancer. Eur Rev Med Pharmacol Sci 17: 618-623, 2013.
28. Xiao F, Zuo Z, Cai G, Kang S, Gao X and Li T: miRecords: An integrated resource for microRNA-target interactions. Nucleic Acids Res 37: D105-D110, 2009.
29. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP and Burge CB: Prediction of mammalian microRNA targets. Cell 115: 787-798, 2003.
30. Betel D, Wilson M, Gabow A, Marks DS and Sander C: The microRNA. org resource: Targets and expression. Nucleic Acids Res 36: D149-D153, 2008.
31. Janky R, Verfaillie A, Imrichová H, Van de Sande B, Standaert L, Christiaens V, Hulselmans G, Herten K, Naval Sanchez M, Potier D, *et al*: iRegulon: from a gene list to a gene regulatory network using large motif and track collections. PLoS Comput Biol 10: e1003731, 2014.
32. Cline MS, Smoot M, Cerami E, Kuchinsky A, Landys N, Workman C, Christmas R, Avila-Campilo I, Creech M, Gross B, *et al*: Integration of biological networks and gene expression data using Cytoscape. Nat Protoc 2: 2366-2382, 2007.
33. Ma L, Liu J, Shen J, Liu L, Wu J, Li W, Luo J, Chen Q and Qian C: Expression of miR-122 mediated by adenoviral vector induces apoptosis and cell cycle arrest of cancer cells. Cancer Biol Ther 9: 554-561, 2010.
34. Raemaekers T, Ribbeck K, Beaudouin J, Annaert W, Van Camp M, Stockmans I, Smets N, Bouillon R, Ellenberg J and Carmeliet G: NuSAP, a novel microtubule-associated protein involved in mitotic spindle organization. J Cell Biol 162: 1017-1029, 2003.
35. Cuzick J, Swanson GP, Fisher G, Brothman AR, Berney DM, Reid JE, Mesher D, Speights VO, Stankiewicz E, Foster CS, *et al*: Prognostic value of an RNA expression signature derived from cell cycle proliferation genes in patients with prostate cancer: A retrospective study. Lancet Oncol 12: 245-255, 2011.
36. Zhou C, Chen H, Han L, Wang A and Chen LA: Identification of featured biomarkers in different types of lung cancer with DNA microarray. Mol Biol Rep 41: 6357-6363, 2014.
37. Pan HW, Chou HY, Liu SH, Peng SY, Liu CL and Hsu HC: Role of L2DTL, cell cycle-regulated nuclear and centrosome protein, in aggressive hepatocellular carcinoma. Cell Cycle 5: 2676-2687, 2006.
38. Li T, Wan B, Huang J and Zhang X: Comparison of gene expression in hepatocellular carcinoma, liver development, and liver regeneration. Mol Genet Genomics 283: 485-492, 2010.
39. Lin ZY, Chuang WL and Chuang YH: Amphotericin B up-regulates angiogenic genes in hepatocellular carcinoma cell lines. Eur J Clin Invest 39: 239-245, 2009.
40. Huang F and Geng XP: Chemokines and hepatocellular carcinoma. World J Gastroenterol 16: 1832-1836, 2010.
41. Schimanski CC, Bahre R, Gockel I, Müller A, Frerichs K, Hörner V, Teufel A, Simiantonaki N, Biesterfeld S, Wehler T, *et al*: Dissemination of hepatocellular carcinoma is mediated via chemokine receptor CXCR4. Br J Cancer 95: 210-217, 2006.
42. Chen Y, Ramjiawan RR, Reiberger T, Ng MR, Hato T, Huang Y, Ochiai H, Kitahara S, Unan EC, Reddy TP, *et al*: CXCR4 inhibition in tumor microenvironment facilitates anti-programmed death receptor-1 immunotherapy in sorafenib-treated hepatocellular carcinoma in mice. Hepatology 61: 1591-1602, 2015.
43. Chew V, Tow C, Teo M, Wong HL, Chan J, Gehring A, Loh M, Bolze A, Quek R, Lee VK, *et al*: Inflammatory tumour microenvironment is associated with superior survival in hepatocellular carcinoma patients. J Hepatol 52: 370-379, 2010.
44. Bandiera S, Pfeffer S, Baumert TF and Zeisel MB: MiR-122-a key factor and therapeutic target in liver disease. J Hepatol 62: 448-457, 2015.
45. Navab R, Strumpf D, Bandarchi B, Zhu CQ, Pintilie M, Ramnarine VR, Ibrahimov E, Radulovich N, Leung L, Barczyk M, *et al*: Prognostic gene-expression signature of carcinoma-associated fibroblasts in non-small cell lung cancer. Proc Natl Acad Sci USA 108: 7160-7165, 2011.
46. Lee HJ, Jang M, Kim H, Kwak W, Park W, Hwang JY, Lee CK, Jang GW, Park MN, Kim HC, *et al*: Comparative transcriptome analysis of adipose tissues reveals that ECM-receptor interaction is involved in the depot-specific adipogenesis in cattle. PLoS One 8: e66267, 2013.
47. Fukui T, Shaykhiev R, Agosto-Perez F, Mezey JG, Downey RJ, Travis WD and Crystal RG: Lung adenocarcinoma subtypes based on expression of human airway basal cell genes. Eur Respir J 42: 1332-1344, 2013.
48. Hou Y: Molecular basis of the effect of midkine on tumor growth in human gastric cancer cell BGC823. Biomedicine and pharmacotherapy 62: 422, 2008.
49. Xia L, Huang W, Tian D, Zhang L, Qi X, Chen Z, Shang X, Nie Y and Wu K: Forkhead box Q1 promotes hepatocellular carcinoma metastasis by transactivating ZEB2 and VersicanV1 expression. Hepatology 59: 958-973, 2014.
50. Hur W, Rhim H, Jung CK, Kim JD, Bae SH, Jang JW, Yang JM, Oh ST, Kim DG, Wang HJ, *et al*: SOX4 overexpression regulates the p53-mediated apoptosis in hepatocellular carcinoma: Clinical implication and functional analysis in vitro. Carcinogenesis 31: 1298-1307, 2010.
51. Liao YL, Sun YM, Chau GY, Chau YP, Lai TC, Wang JL, Horng JT, Hsiao M and Tsou AP: Identification of SOX4 target genes using phylogenetic footprinting-based prediction from expression microarrays suggests that overexpression of SOX4 potentiates metastasis in hepatocellular carcinoma. Oncogene 27: 5578-5589, 2008.
52. Chang Q, Chen J, Beezhold KJ, Castranova V, Shi X and Chen F: JNK1 activation predicts the prognostic outcome of the human hepatocellular carcinoma. Mol Cancer 8: 1-14, 2009.
53. Elyakim E, Sitbon E, Faerman A, Tabak S, Montia E, Belanis L, Dov A, Marcusson EG, Bennett CF, Chajut A, *et al*: hsa-miR-191 is a candidate oncogene target for hepatocellular carcinoma therapy. Cancer Res 70: 8077-8087, 2010.