SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 15: 9625-9632, 2018

Screening therapeutic targets of ribavirin in hepatocellular carcinoma

CHEN XU[1*], LIYUN LUO[2*], YONGJUN YU[1], ZHAO ZHANG[1], YI ZHANG[1], HAIMEI LI[1], YUE CHENG[1], HAI QIN[1], XIPENG ZHANG[1], HONGMEI MA[3] and YUWEI LI[1]

[1]Department of Colorectal Surgery, Tianjin Union Medical Center, Tianjin 300121;
[2]Department of Cardiology, The Fifth Affiliated Hospital of Sun Yan-Sen University, Zhuhai, Guangdong 519000;
[3]Department of Nursing, Tianjin Union Medical Center, Tianjin 300121, P.R. China

Received December 28, 2016; Accepted October 13, 2017

DOI: 10.3892/ol.2018.8552

Abstract. The objective of the present study was to screen the key genes of ribavirin in hepatocellular carcinoma (HCC) and provide novel therapeutic targets for HCC treatment. The mRNA expression datasets of GSE23031 and GSE74656, as well as the microRNA (miRNA) expression dataset of GSE22058 were downloaded from the Gene Expressed Omnibus database. In the GSE23031 dataset, there were three HCC cell lines treated with PBS and three HCC cell lines treated with ribavirin. In the GSE74656 dataset, five HCC tissues and five carcinoma adjacent tissues were selected. In the GSE22058 dataset, 96 HCC tissues and 96 carcinoma adjacent tissues were selected. The differentially expressed genes (DEGs) and differentially expressed miRNAs were identified via the limma package of *R*. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed with the Database for Annotation, Visualization and Integrated Discovery. The target mRNAs of DEMs were obtained with TargetScan. A total of 559 DEGs (designated DEG-Ribavirin) were identified in HCC cells treated with ribavirin compared with PBS and 632 DEGs (designated DEG-Tumor) were identified in HCC tissues compared with carcinoma adjacent tissues. A total of 220 differentially expressed miRNAs were identified in HCC tissues compared with carcinoma adjacent tissues. In addition, 121 GO terms and three KEGG pathways of DEG-Ribavirin were obtained, and 383 GO terms and 25 KEGG pathways of DEG-Tumor were obtained. A total of five key miRNA-mRNA regulated pairs were identified, namely *miR-183→CCNB1, miR-96→DEPDC1, miR-96→NTN4, miR-183→NTN4* and *miR-145→NTN4*. The present study indicated that certain miRNAs (including *miR-96, miR-145* and *miR-183*) and mRNAs (including *NAT2, FBXO5, CCNB1, DEPDC1* and *NTN4*) may be associated with the effects of ribavirin on HCC. Furthermore, they may provide novel therapeutic targets for HCC treatment.

Correspondence to: Dr Hongmei Ma, Department of Nursing, Tianjin Union Medical Center, 190 Jieyuan Road, Hongqiao, Tianjin 300121, P.R. China
E-mail: strawberry2046@163.com

Dr Yuwei Li, Department of Colorectal Surgery, Tianjin Union Medical Center, 190 Jieyuan Road, Hongqiao, Tianjin 300121, P.R. China
E-mail: xc198129@163.com

*Contributed equally

Key words: hepatocellular carcinoma, ribavirin, microarray analysis

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and arises most frequently in patients with cirrhosis (1). It is the second most common cause of cancer-associated mortality globally with 1.6 million mortalities per year, and it is hypothesized that the high global incidence rate and late presentation of HCC may be responsible for this (2,3). Additionally, the general prognosis was poor with an overall survival rate between 3 and 5% in 2006 (4). Symptoms of HCC include yellow skin, bloating from fluid in the abdomen, easy bruising from blood clotting abnormalities, loss of appetite, unintentional weight loss, nausea, vomiting and tiredness (5,6). The primary risk factors for HCC were hepatitis C, hepatitis B, alcoholism, aflatoxin and cirrhosis of the liver (7-10). Liver transplantation, tyrosine kinase inhibitors and surgical resection are currently the primary treatment options (11-13). The treatment of HCC has not been fundamentally improved, which may be seen in the increasing morbidity and mortality each year (14). Ribavirin is an anti-viral drug used to treat hepatitis C, respiratory syncytial virus and other viral infections. If infection is persistent, ribavirin is often used in combination with peginterferon α-2b or peginterferon α-2a (15,16). It has been reported that hepatitis C infection was globally associated with 25% of HCC cases in 2006 (15). Therefore, ribavirin, by itself or in conjunction with peginterferon α-2b or pegylated interferon, has been used to treat HCC in patients with viral infections (17-20). Exploration of the genetic changes in HCC cells is necessary for the study of the pathogenesis and progression of HCC, as well as to develop effective treatments. In the present study, a microarray analysis

of mRNA and microRNA (miRNA) was performed in the treatment of ribavirin on HCC, in order to identify possible biomarkers and provide novel potential therapeutic targets for HCC.

Materials and methods

Microarray data and data prx10-processing. The mRNA expression datasets of GSE23031 and GSE74656, as well as the miRNA expression dataset of GSE22058, (21-23) were downloaded from the Gene Expressed Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). They were analyzed using the platforms GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array (Thermo Fisher Scientific, Inc., Waltham, MA, USA), GPL16043 GeneChip® PrimeView™ Human Gene Expression Array (with External spikx10-in RNAs; Thermo Fisher Scientific, Inc.) and GPL10457 Rosetta human miRNA qPCR array (Rosetta Inpharmatics; Merck Sharp & Dohme, Hoddesdon, UK), respectively. The mRNA data (GSE23031) contained three HCC cell lines treated with PBS and three HCC cell lines treated with ribavirin. In the GSE74656, five HCC tissues and five carcinoma adjacent tissues were selected for the study. In the GSE22058, 96 HCC tissues and 96 carcinoma adjacent tissues were selected to study. Robust Multi-Array Average (RMA) was an algorithm used to create an expression matrix from Affymetrix data (24). The raw data were converted into a recognizable format by *R*, and the RMA was used for correction and normalization.

Differential expression analysis. The differentially expressed genes (DEGs) were identified via the limma package V3.32.10 (http://www.bioconductor.org/packages/3.5/bioc/html/limma.html) (25). According to the criteria: $P<0.05$ and $|\log(\text{fold change})|>1$, the DEGs were identified in HCC cells treated with ribavirin compared with PBS and designated DEG-Ribavirin. With the same criteria, the DEGs were identified in HCC tissues compared with their matched adjacent tissues and designated DEG-Tumor. Additionally, the differentially expressed miRNAs (DEMs) were obtained in HCC tissues compared with carcinoma adjacent tissues with $P<0.05$ and $|\log(\text{fold change})|>0.3$.

Functional and pathway enrichment analysis. The Database for Annotation, Visualization and Integrated Discovery (https://david.ncifcrf.gov/) (26) is a widely-used web-based tool for functional and pathway enrichment analysis. In the present study, it was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEG-Ribavirin and DEG-Tumor data. The GO terms and KEGG pathways were selected with $P<0.05$.

Comparison of DEGs and screening of miRNA-mRNA regulated pairs. The overlapped DEGs of DEG-Ribavirin and DEG-Tumor were selected, and the overlapped DEGs with opposite expression between DEG-Ribavirin and DEG-Tumor were also selected. The TargetScan database was used to predict biological target mRNAs of miRNAs that matched the seed region of each miRNA (27). The target mRNAs of DEMs were then selected using TargetScan. The key miRNAs, which regulated the overlapped DEGs with opposite expression between DEG-Ribavirin and DEG-Tumor, were identified. Subsequently, the miRNA-mRNA regulated pairs were constructed.

Table I. Top 30 most significant differentially expressed genes in hepatocellular carcinoma cells treated with ribavirin compared with PBS.

Gene	logFC	Average expression	P-value
NCF2	3.636237	10.08812	4.01×10^{-13}
TXNIP	3.04897	9.867782	5.95×10^{-12}
PRORSD1P	2.527183	6.729178	4.91×10^{-11}
NPPB	3.0072	12.09607	8.26×10^{-11}
CYR61	2.018572	10.7489	9.19×10^{-11}
PLA2G4C	1.93915	5.61348	9.29×10^{-11}
CDKN2B	2.282063	7.523593	9.52×10^{-11}
LEAP2	2.52559	9.937438	1.39×10^{-10}
DUSP4	1.86831	8.419143	3.18×10^{-10}
FLVCR1-AS1	2.01682	8.90536	4.17×10^{-10}
ASH1L-AS1	1.803117	9.666342	4.20×10^{-10}
CXCL3	1.797497	8.092698	5.80×10^{-10}
GLIPR2	1.688522	9.089844	5.91×10^{-10}
CYP1A1	1.560867	11.576	7.61×10^{-10}
EID2B	1.667057	8.101158	9.18×10^{-10}
JUNB	1.574027	8.65205	1.10×10^{-09}
BTG2	1.498662	9.091506	1.41×10^{-09}
PPL	1.94965	8.769882	2.08×10^{-09}
ZNF436-AS1	2.570947	8.267133	2.12×10^{-09}
TIGD7	1.654807	6.61165	2.17×10^{-09}
LOC284513	1.59588	6.487373	2.40×10^{-09}
TUFT1	1.50993	10.31677	2.43×10^{-09}
CTSE	1.439267	11.22817	2.48×10^{-09}
ERP27	1.376543	10.6632	2.51×10^{-09}
UCA1	1.611053	10.73581	2.60×10^{-09}
HSD3B1	1.699597	5.372037	2.61×10^{-09}
GATA6-AS1	1.971443	9.336978	2.99×10^{-09}
LOC100134822	1.45604	7.840603	3.38×10^{-09}
THUMPD3-AS1	1.365701	7.84944	3.41×10^{-09}
GDA	1.482742	8.376296	3.62×10^{-09}

logFC, log fold-change.

Results

DEGs. A total of 559 DEGs (269 upregulated and 290 downregulated) and 623 DEGs (272 upregulated and 351 downregulated) were identified in DEG-Ribavirin and DEG-Tumor. The heat map of them and the top 30 most significant DEGs are presented in Figs. 1 and 2, Tables I and II, respectively. A total of 220 DEMs were obtained. The 30 most significant DEMs are presented in Table III.

GO terms and KEGG pathways. A total of 121 GO terms and 3 KEGG pathways (cell cycle pathway, p53 signaling pathway and glycine, serine and threonine metabolism pathway) of

Table II. Top 30 most significant DEGs in HCC tissues compared with carcinoma adjacent tissues.

Gene	logFC	Average expression	P-value
CTHRC1	2.856945	5.825291	$6.69x10^{-07}$
PEA15	1.468145	8.69977	$1.04x10^{-06}$
CENPE	2.043658	6.056165	$1.29x10^{-06}$
C21orf56	1.237448	5.670084	$1.54x10^{-06}$
DBN1	1.383559	6.53977	$1.76x10^{-06}$
GLA	1.380323	7.172235	$2.00x10^{-06}$
DDX39	1.17872	7.721654	$2.63x10^{-06}$
MPV17	1.190142	9.079669	$2.67x10^{-06}$
RFX5	1.417855	7.558042	$2.89x10^{-06}$
TMEM144	1.379456	5.655336	$3.59x10^{-06}$
ASNS	2.515006	6.603396	$3.63x10^{-06}$
SLC38A6	1.678498	7.591919	$3.93x10^{-06}$
GRAMD1A	1.013492	7.228709	$4.65x10^{-06}$
COMMD8	1.109678	8.353807	$7.57x10^{-06}$
YWHAZ	1.008572	8.92205	$7.94x10^{-06}$
PLXNC1	1.451381	5.772042	$1.18x10^{-05}$
PLVAP	1.001818	5.796379	$1.20x10^{-05}$
SHCBP1	1.327673	4.917339	$1.39x10^{-05}$
LAMC1	1.104793	6.905647	$1.46x10^{-05}$
ANXA2P2	1.89594	11.61567	$1.52x10^{-05}$
ACTR3	1.094921	9.606995	$1.63x10^{-05}$
CCDC88A	1.002649	6.195715	$1.64x10^{-05}$
E2F3	1.093946	6.541921	$1.75x10^{-05}$
FAM118B	1.006248	6.9915	$1.76x10^{-05}$
ZNF354A	1.069078	5.918941	$1.81x10^{-05}$
RASGEF1A	2.021872	4.908126	$1.84x10^{-05}$
NUP37	1.362635	6.839194	$2.01x10^{-05}$
ESM1	1.291572	4.762991	$2.03x10^{-05}$
KPNA2	2.606398	9.341649	$2.13x10^{-05}$
DCUN1D5	1.38889	8.463629	$2.34x10^{-05}$

logFC, log fold-change; HCC, hepatocellular carcinoma; DEGs, differentially expressed genes.

Table III. Top 30 most significant differentially expressed miRNA in HCC tissues compared with carcinoma adjacent tissues.

miRNA	logFC	P-value
hsa-mir-188	0.36842	$2.41x10^{-39}$
hsa-mir-106b	0.32071	$1.52x10^{-36}$
hsa-mir-214	-0.54861	$7.34x10^{-36}$
hsa-mir-93	0.32346	$1.73x10^{-35}$
hsa-mir-10a	-0.51702	$6.33x10^{-35}$
hsa-mir-199a-1	-0.78765	$6.28x10^{-33}$
hsa-mir-199a-2	-0.73035	$5.57x10^{-31}$
hsa-mir-301	0.5629	$1.16x10^{-28}$
hsa-mir-424	-0.31246	$1.72x10^{-28}$
hsa-mir-33	0.29308	$7.92x10^{-26}$
hsa-mir-324-5p	0.37868	$1.01x10^{-23}$
hsa-mir-25	0.20282	$1.53x10^{-22}$
hsa-mir-125b	-0.37741	$1.95x10^{-22}$
hsa-mir-339	0.21825	$3.36x10^{-21}$
hsa-mir-145	-0.35693	$4.27x10^{-21}$
hsa-mir-148b	0.20955	$6.76x10^{-21}$
hsa-mir-151	0.25728	$9.53x10^{-21}$
hsa-mir-221	0.35829	$1.39x10^{-20}$
hsa-mir-18a	0.41731	$3.09x10^{-20}$
hsa-mir-130b	0.38724	$3.75x10^{-20}$
hsa-mir-195	-0.34663	$6.72x10^{-20}$
hsa-mir-99a	-0.38524	$8.55x10^{-20}$
hsa-mir-15b	0.27273	$1.80x10^{-19}$
hsa-mir-183	0.42758	$2.31x10^{-19}$
hsa-mir-222	0.30098	$2.62x10^{-18}$
hsa-mir-125a	-0.26924	$4.47x10^{-18}$
hsa-mir-378	-0.33767	$7.60x10^{-18}$
hsa-mir-101	-0.24801	$1.85x10^{-17}$
hsa-mir-331	0.20462	$2.33x10^{-17}$
hsa-mir-200b	-0.6996	$2.38x10^{-17}$

HCC, hepatocellular carcinoma; logFC, log fold-change; mir, microRNA.

DEG-Ribavirin were obtained. A total of 383 GO terms and 25 KEGG pathways of DEG-Tumor were obtained. The top 20 enriched GO terms of DEG-Ribavirin and DEG-Tumor are presented in Tables IV and V, respectively. The enriched KEGG pathways of DEG-Ribavirin and DEG-Tumor are presented in Tables VI and VII, respectively.

miRNA-mRNA regulated pairs. There were 50 overlapped DEGs, with 32 [including N-acetyltransferase (*NAT2*) and F-box only protein 5 (*FBXO5*)] exhibiting opposite expression between DEG-Ribavirin, and DEG-Tumor. A heat map of the 32 overlapped DEGs is presented in Fig. 3. Furthermore, three DEMs (*miR-96, miR-145* and *miR-183*) were revealed to correspond to three DEGs (*CCNB1, DEPDC1* and *NTN4*) which were included in the aforementioned 32 overlapped DEGs. Finally, five miRNA-mRNA regulated pairs were selected between the above three DEGs and the three DEMs, namely *miR-183→CCNB1, miR-96→DEPDC1, miR-96→NTN4, miR-183→NTN4* and *miR-145→NTN4*.

Discussion

In the present study, the DEGs in HCC cells treated with ribavirin compared with PBS treated HCC tissue, HCC tissues and carcinoma adjacent tissues, were firstly identified, and 32 overlapped DEGs with opposite expression between DEG-Ribavirin and DEG-Tumor were selected. It was notable that *NAT2* and *FBXO5* were two mRNAs of them with opposite expression between DEG-Ribavirin and DEG-Tumor. NAT2 serves a function in the metabolic activation and detoxification of aromatic amines, which in turn serves a function in the metabolism of aromatic and heterocyclic amines, and hydrazines via N-acetylation and O-acetylation (28). As early as in

Table IV. Top 20 enriched GO terms of DEG-Ribavirin.

Category	GO ID	Go name	Gene number	P-value
BP	GO:0022403	Cell cycle phase	29	$1.66x10^{-06}$
BP	GO:0007049	Cell cycle	43	$1.66x10^{-06}$
BP	GO:0000279	M phase	24	$8.49x10^{-06}$
BP	GO:0007067	Mitosis	19	$1.02x10^{-05}$
BP	GO:0000280	Nuclear division	19	$1.02x10^{-05}$
BP	GO:0000087	M phase of mitotic cell cycle	19	$1.29x10^{-05}$
BP	GO:0048285	Organelle fission	19	$1.73x10^{-05}$
BP	GO:0000278	Mitotic cell cycle	25	$1.88x10^{-05}$
BP	GO:0022402	Cell cycle process	32	$3.23x10^{-05}$
BP	GO:0008283	Cell proliferation	27	$3.62x10^{-05}$
BP	GO:0031497	Chromatin assembly	11	$5.88x10^{-05}$
BP	GO:0065004	Protein-DNA complex assembly	11	$8.65x10^{-05}$
BP	GO:0006334	Nucleosome assembly	10	$2.36x10^{-04}$
BP	GO:0051726	Regulation of cell cycle	21	$2.43x10^{-04}$
BP	GO:0051301	Cell division	19	$4.39x10^{-04}$
BP	GO:0034728	Nucleosome organization	10	$5.08x10^{-04}$
BP	GO:0006323	DNA packaging	11	$6.79x10^{-04}$
CC	GO:0000786	Nucleosome	8	$9.25x10^{-04}$
BP	GO:0010033	Response to organic substance	33	0.001109
CC	GO:0005819	Spindle	12	0.001114

GO, Gene Ontology; DEGs, differentially expressed genes; CC, cellular component; BP, biological process.

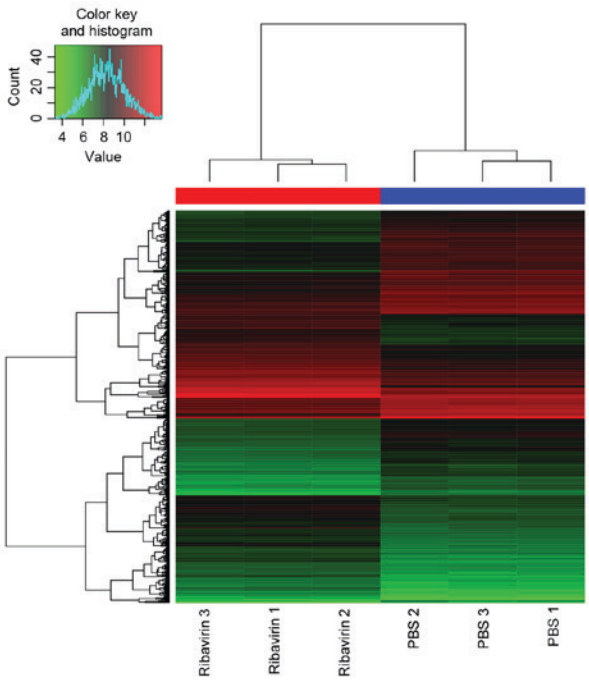

Figure 1. Heatmap of differentially expressed genes in hepatocellular carcinoma cells treated with ribavirin compared with PBS.

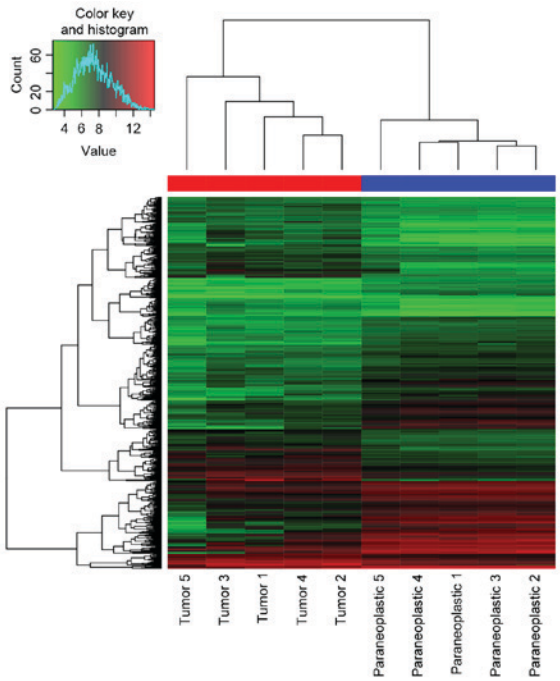

Figure 2. Heatmap of differentially expressed genes in hepatocellular carcinoma tissues compared with their matched adjacent tissues.

1996, Agúndez *et al* (29) reported that the slow acetylation was associated with an increased risk of HCC. Furthermore, it has been demonstrated that *NAT2* activity is associated with smoking-associated HCC (30-32). A number of previous studies that have investigated the association between *NAT2* genotypes and HCC risk have been published (32-36). *FBXO5*, also known as early mitotic inhibitor-1, is a key cell-cycle regulator that promotes S-phase and M-phase entry by inhibiting

Table V. Top 20 enriched GO terms of DEG-tumor.

Category	GO ID	Go name	Gene number	P-value
BP	GO:0000279	M phase	49	$1.22x10^{-17}$
BP	GO:0022403	Cell cycle phase	54	$7.41x10^{-17}$
BP	GO:0007067	Mitosis	39	$1.19x10^{-16}$
BP	GO:0000280	Nuclear division	39	$1.19x10^{-16}$
BP	GO:0000087	M phase of mitotic cell cycle	39	$2.21x10^{-16}$
BP	GO:0000278	Mitotic cell cycle	50	$4.09x10^{-16}$
BP	GO:0048285	Organelle fission	39	$5.79x10^{-16}$
BP	GO:0022402	Cell cycle process	61	$5.37x10^{-15}$
MF	GO:0048037	Cofactor binding	39	$3.37x10^{-14}$
BP	GO:0016054	Organic acid catabolic process	26	$4.73x10^{-14}$
BP	GO:0046395	Carboxylic acid catabolic process	26	$4.73x10^{-14}$
CC	GO:0005819	Spindle	30	$7.20x10^{-14}$
BP	GO:0007049	Cell cycle	71	$9.42x10^{-14}$
BP	GO:0055114	Oxidation reduction	62	$3.81x10^{-13}$
BP	GO:0007059	Chromosome segregation	21	$2.69x10^{-12}$
MF	GO:0009055	Electron carrier activity	34	$3.23x10^{-12}$
CC	GO:0000793	Condensed chromosome	26	$5.97x10^{-12}$
CC	GO:0000777	Condensed chromosome kinetochore	18	$1.41x10^{-11}$
MF	GO:0050662	Coenzyme binding	29	$6.32x10^{-11}$
CC	GO:0000779	Condensed chromosome, centromeric region	18	$1.37x10^{-10}$

GO, Gene Ontology; DEGs, differentially expressed genes; CC, cellular component; BP, biological process; MF, molecular foundation.

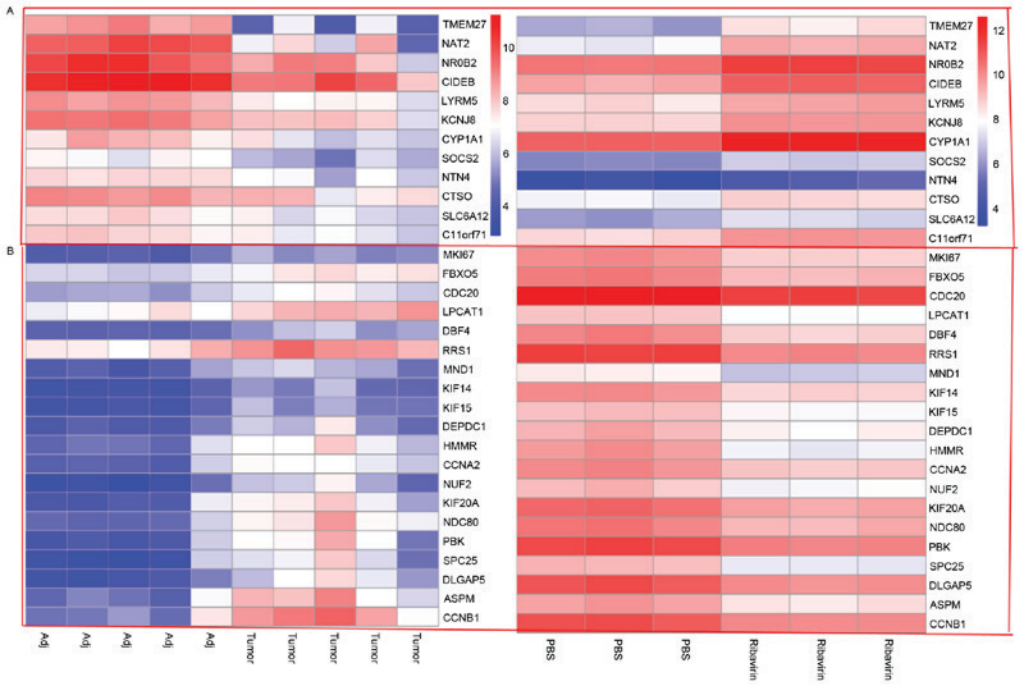

Figure 3. Heatmap of the overlapped DEGs with opposite expression between DEG-Ribavirin and DEG-Tumor. Adj, adjacent; DEG, differentially expressed gene.

anaphasx10-promoting complex/cyclosome activity (37). Zhao *et al* (38) revealed that *FBXO5* was overexpressed in HCC, which is in agreement with the results of the present study, and also reported that *FBXO5* may control tumor cell proliferation in HCC. In the present study, it was identified that the expression of *NAT2* was lower in HCC cells and HCC tissues. However, expression was increased following treatment with ribavirin. However, *FBXO5* was overexpressed in HCC

Table VI. Enriched KEGG pathways of DEG-Ribavirin.

Category	Pathway name	Gene number	P-value
KEGG_PATHWAY	hsa04110: Cell cycle	15	1.19×10^{-05}
KEGG_PATHWAY	hsa00260: Glycine, serine and threonine metabolism	5	0.011495
KEGG_PATHWAY	hsa04115: p53 signaling pathway	6	0.045851

DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table VII. Enriched KEGG pathways of DEG-tumor.

Category	Pathway name	Gene number	P-value
KEGG_PATHWAY	hsa00071: Fatty acid metabolism	15	1.37×10^{-09}
KEGG_PATHWAY	hsa00280: Valine, leucine and isoleucine degradation	14	5.58×10^{-08}
KEGG_PATHWAY	hsa04110: Cell cycle	21	1.08×10^{-06}
KEGG_PATHWAY	hsa00830: Retinol metabolism	12	3.12×10^{-05}
KEGG_PATHWAY	hsa00380: Tryptophan metabolism	10	7.32×10^{-05}
KEGG_PATHWAY	hsa00650: Butanoate metabolism	9	1.33×10^{-04}
KEGG_PATHWAY	hsa00250: Alanine, aspartate and glutamate metabolism	8	4.63×10^{-04}
KEGG_PATHWAY	hsa04114: Oocyte meiosis	15	5.67×10^{-04}
KEGG_PATHWAY	hsa00640: Propanoate metabolism	8	5.69×10^{-04}
KEGG_PATHWAY	hsa03320: PPAR signaling pathway	11	0.001281
KEGG_PATHWAY	hsa00980: Metabolism of xenobiotics by cytochrome P450	10	0.00174
KEGG_PATHWAY	hsa00910: Nitrogen metabolism	6	0.003688
KEGG_PATHWAY	hsa00590: Arachidonic acid metabolism	9	0.004249
KEGG_PATHWAY	hsa00140: Steroid hormone biosynthesis	8	0.005165
KEGG_PATHWAY	hsa00982: Drug metabolism	9	0.007941
KEGG_PATHWAY	hsa00591: Linoleic acid metabolism	6	0.008896
KEGG_PATHWAY	hsa00340: Histidine metabolism	6	0.010346
KEGG_PATHWAY	hsa04115: p53 signaling pathway	9	0.013645
KEGG_PATHWAY	hsa00260: Glycine, serine and threonine metabolism	6	0.013718
KEGG_PATHWAY	hsa00410: β-Alanine metabolism	5	0.017838
KEGG_PATHWAY	hsa04920: Adipocytokine signaling pathway	8	0.036604
KEGG_PATHWAY	hsa00620: Pyruvate metabolism	6	0.037809
KEGG_PATHWAY	hsa00232: Caffeine metabolism	3	0.039507
KEGG_PATHWAY	hsa05222: Small cell lung cancer	9	0.042544
KEGG_PATHWAY	hsa04512: ECM-receptor interaction	9	0.042544

DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

cells and HCC tissues, and decreased following treatment with ribavirin. Therefore, it is suspected that *NAT2* and *FBXO5* may be biomarkers of ribavirin in the treatment of HCC.

The cell cycle has been demonstrated to be associated with the progression and migration of HCC (39-41), and regulation of the cell cycle is considered an effective strategy for HCC treatment (42-45). The p53 signaling pathway has been heavily studied and is reported to serve a function in the occurrence and development of HCC (45-49). The association between the glycine, serine and threonine metabolism pathway and HCC has been less studied, and the glycine, serine and threonine metabolism pathway was also enriched in DEG-Tumor tissues. In this study, only three KEGG pathways of DEG-Ribavirin were obtained, namely cell cycle, p53 signaling pathway and glycine, serine and threonine metabolism. Cell cycle was the most significantly enriched function in this study, which was identified from the enriched GO terms of DEG-Ribavirin (e.g. cell cycle phase, cell cycle and M phase) and DEG-Tumor (e.g. M phase and cell cycle phase), as well as the enriched KEGG pathways of DEG-Tumor. The results of the present study suggest that these three KEGG pathways may be associated with the pathogenesis and treatment of HCC; however, more in-depth research is required.

In the present study, three DEMs (*miR-96, miR-145* and *miR-183*) were identified to correspond to three overlapped DEGs (*CCNB1, DEPDC1* and *NTN4*) with opposite expression

in DEG-Ribavirin and DEG-Tumor, and 5 miRNA-mRNA regulated pairs were selected, namely *miR-183→CCNB1, miR-96→DEPDC1, miR-96→NTN4, miR-183→NTN4* and *miR-145→NTN4*. It has been demonstrated that *miR-96* down-regulation may suppress the growth of HCC (50), and *miR-96* may promote cell proliferation and invasion through targeting ephrinA5 in HCC (51). Chen *et al* (52) considered serum *miR-96* as a promising biomarker for HCC with chronic hepatitis B virus infection. Previous studies have demonstrated that *miR-145* may inhibit proliferation, migration and invasion, as well as promote apoptosis in HCC (53-56). *MiR-183* may also regulate the growth, invasion and apoptosis of HCC (57-59). It has been identified that these miRNA and mRNA are possible biomarkers of ribavirin in HCC, and they may regulate HCC through the 5 miRNA-mRNA pairs.

In conclusion, a number of miRNAs (e.g. *miR-96, miR-145* and *miR-183*) and mRNAs (e.g. *NAT2, FBXO5, CCNB1, DEPDC1* and *NTN4*) may be associated with the effects of ribavirin on HCC. Furthermore, they may provide novel therapeutic targets for drugs of HCC.

References

1. El-Serag HB: Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology 142: 1264-1273.e1, 2012.
2. McGuire S: World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. Adv Nutr 7: 418-419, 2016.
3. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136: E359-E386, 2015.
4. Lopez PM, Villanueva A and Llovet JM: Systematic review: Evidencx10-based management of hepatocellular carcinoma-an updated analysis of randomized controlled trials. Aliment Pharmacol Ther 23: 1535-1547, 2006.
5. Kaiser K, Mallick R, Butt Z, Mulcahy MF, Benson AB and Cella D: Important and relevant symptoms including pain concerns in hepatocellular carcinoma (HCC): A patient interview study. Support Care Cancer 22: 919-926, 2014.
6. Ji Z, Meng G, Huang D, Yue X and Wang B: NMFBFS: A NMF-based feature selection method in identifying pivotal clinical symptoms of hepatocellular carcinoma. Comput Math Methods Med 2015: 846942, 2015.
7. Paul SB, Shalimar, Sreenivas V, Gamanagatti SR, Sharma H, Dhamija E and Acharya SK: Incidence and risk factors of hepatocellular carcinoma in patients with hepatic venous outflow tract obstruction. Aliment Pharmacol Ther 41: 961-971, 2015.
8. Rong G, Wang H, Bowlus CL, Wang C, Lu Y, Zeng Z, Qu J, Lou M, Chen Y, An L, *et al*: Incidence and risk factors for hepatocellular carcinoma in primary biliary cirrhosis. Clin Rev Allergy Immunol 48: 132-141, 2015.
9. Toyoda H, Kumada T, Tada T, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, Kitabatake S and Ito T: Risk factors of hepatocellular carcinoma development in non-cirrhotic patients with sustained virologic response for chronic hepatitis C virus infection. J Gastroenterol Hepatol 30: 1183-1189, 2015.
10. Zhang XX, Wang LF, Jin L, Li YY, Hao SL, Shi YC, Zeng QL, Li ZW, Zhang Z, Lau GK and Wang FS: Primary biliary cirrhosis-associated hepatocellular carcinoma in Chinese patients: Incidence and risk factors. World J Gastroenterol 21: 3554-3563, 2015.
11. Sacco R, Gadaleta-Caldarola G, Galati G, Lombardi G, Mazza G and Cabibbo G: EASL HCC summit: Liver cancer management. Future Oncol 10: 1129-1132, 2014.
12. Fitzmorris P, Shoreibah M, Anand BS and Singal AK: Management of hepatocellular carcinoma. J Cancer Res Clin Oncol 141: 861-876, 2015.
13. Park JW, Chen M, Colombo M, Roberts LR, Schwartz M, Chen PJ, Kudo M, Johnson P, Wagner S, Orsini LS and Sherman M: Global patterns of hepatocellular carcinoma management from diagnosis to death: The BRIDGE Study. Liver Int 35: 2155-2166, 2015.
14. Scaggiante B, Farra R, Dapas B, Baj G, Pozzato G, Grassi M, Zanconati F and Grassi G: Aptamer targeting of the elongation factor 1A impairs hepatocarcinoma cells viability and potentiates bortezomib and idarubicin effects. Int J Pharm 506: 268-279, 2016.
15. Alter MJ: Epidemiology of hepatitis C virus infection. World J Gastroenterol 13: 2436-2441, 2007.
16. Smith DW, Frankel LR, Mathers LH, Tang AT, Ariagno RL and Prober CG: A controlled trial of aerosolized ribavirin in infants receiving mechanical ventilation for severe respiratory syncytial virus infection. N Engl J Med 325: 24-29, 1991.
17. Harada N, Hiramatsu N, Oze T, Morishita N, Yamada R, Hikita H, Miyazaki M, Yakushijin T, Miyagi T, Yoshida Y, *et al*: Risk factors for hepatocellular carcinoma in hepatitis C patients with normal alanine aminotransferase treated with pegylated interferon and ribavirin. J Viral Hepat 21: 357-365, 2014.
18. Liu CJ, Chu YT, Shau WY, Kuo RN, Chen PJ and Lai MS: Treatment of patients with dual hepatitis C and B by peginterferon alpha and ribavirin reduced risk of hepatocellular carcinoma and mortality. Gut 63: 506-514, 2014.
19. Honda T, Ishigami M, Masuda H, Ishizu Y, Kuzuya T, Hayashi K, Itoh A, Hirooka Y, Nakano I, Ishikawa T, *et al*: Effect of peginterferon alfa-2b and ribavirin on hepatocellular carcinoma prevention in older patients with chronic hepatitis C. J Gastroenterol Hepatol 30: 321-328, 2015.
20. Krastev Z, Jelev D, Antonov K, Petkova T, Atanasova E, Zheleva N, Tomov B, Boyanova Y and Mateva L: Ombitasvir, paritaprevir, ritonavir, dasabuvir and ribavirin in cirrhosis after complete destruction of hepatocellular carcinoma. World J Gastroenterol 22: 2630-2635, 2016.
21. Thomas E, Feld JJ, Li Q, Hu Z, Fried MW and Liang TJ: Ribavirin potentiates interferon action by augmenting interferon-stimulated gene induction in hepatitis C virus cell culture models. Hepatology 53: 32-41, 2011.
22. Burchard J, Zhang C, Liu AM, Poon RT, Lee NP, Wong KF, Sham PC, Lam BY, Ferguson MD, Tokiwa G, *et al*: microRNA-122 as a regulator of mitochondrial metabolic gene network in hepatocellular carcinoma. Mol Syst Biol 6: 402, 2010.
23. Liu AM, Yao TJ, Wang W, Wong KF, Lee NP, Fan ST, Poon RT, Gao C and Luk JM: Circulating miR-15b and miR-130b in serum as potential markers for detecting hepatocellular carcinoma: A retrospective cohort study. BMJ Open 2: e000825, 2012.
24. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U and Speed TP: Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4: 249-264, 2003.
25. Diboun I, Wernisch L, Orengo CA and Koltzenburg M: Microarray analysis after RNA amplification can detect pronounced differences in gene expression using limma. BMC Genomics 7: 252, 2006.
26. 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.
27. Lewis BP, Burge CB and Bartel DP: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120: 15-20, 2005.
28. Smith CA, Smith G and Wolf CR: Genetic polymorphisms in xenobiotic metabolism. Eur J Cancer 30A: 1921-1935, 1994.
29. Agundez JA, Olivera M, Ladero JM, Rodriguez-Lescure A, Ledesma MC, Diaz-Rubio M, Meyer UA and Benítez J: Increased risk for hepatocellular carcinoma in NAT2-slow acetylators and CYP2D6-rapid metabolizers. Pharmacogenetics 6: 501-512, 1996.
30. Farker K, Schotte U, Scheele J and Hoffmann A: Impact of N-acetyltransferase polymorphism (NAT2) in hepatocellular carcinoma (HCC)-an investigation in a department of surgical medicine. Exp Toxicol Pathol 54: 387-391, 2003.
31. Zhang J, Xu F and Ouyang C: Joint effect of polymorphism in the N-acetyltransferase 2 gene and smoking on hepatocellular carcinoma. Tumour Biol 33: 1059-1063, 2012.
32. Farker K, Schotte U, Scheele J and Hoffmann A: Assessment of frequencies of lifestyle factors and polymorphisms of drug-metabolizing enzymes (NAT2, CYP2E1) in human hepatocellular carcinoma (HCC) patients in a department of surgical medicinx10-a pilot investigation. Int J Clin Pharmacol Ther 40: 120-124, 2002.
33. Huang YS, Chern HD, Wu JC, Chao Y, Huang YH, Chang FY and Lee SD: Polymorphism of the N-acetyltransferase 2 gene, red meat intake, and the susceptibility of hepatocellular carcinoma. Am J Gastroenterol 98: 1417-1422, 2003.

34. Blum HE: Hepatocellular carcinoma: Susceptibility markers. IARC Sci Publ 154: 241-244, 2001.
35. Gelatti U, Covolo L, Talamini R, Tagger A, Barbone F, Martelli C, Cremaschini F, Franceschi S, Ribero ML, Garte S, *et al*: N-Acetyltransferasx10-2, glutathione S-transferase M1 and T1 genetic polymorphisms, cigarette smoking and hepatocellular carcinoma: A casx10-control study. Int J Cancer 115: 301-306, 2005.
36. Xu B, Wang F, Song C, Sun Z, Cheng K, Tan Y, Wang H and Zou H: Largx10-scale proteome quantification of hepatocellular carcinoma tissues by a three-dimensional liquid chromatography strategy integrated with sample preparation. J Proteome Res 13: 3645-3654, 2014.
37. Gütgemann I, Lehman NL, Jackson PK and Longacre TA: Emi1 protein accumulation implicates misregulation of the anaphase promoting complex/cyclosome pathway in ovarian clear cell carcinoma. Mod Pathol 21: 445-454, 2008.
38. Zhao Y, Tang Q, Ni R, Huang X, Wang Y, Lu C, Shen A, Wang Y, Li C, Yuan Q, *et al*: Early mitotic inhibitor-1, an anaphasx10-promoting complex/cyclosome inhibitor, can control tumor cell proliferation in hepatocellular carcinoma: Correlation with Skp2 stability and degradation of p27(Kip1). Human Pathol 44: 365-373, 2013.
39. Feng YM, Feng CW, Chen SY, Hsieh HY, Chen YH and Hsu CD: Cyproheptadine, an antihistaminic drug, inhibits proliferation of hepatocellular carcinoma cells by blocking cell cycle progression through the activation of P38 MAP kinase. BMC Cancer 15: 134, 2015.
40. Wang Z, Wei W, Sun CK, Chua MS and So S: Suppressing the CDC37 cochaperone in hepatocellular carcinoma cells inhibits cell cycle progression and cell growth. Liver Int 35: 1403-1415, 2015.
41. Deng L, Yang J, Chen H, Ma B, Pan K, Su C, Xu F and Zhang J: Knockdown of TMEM16A suppressed MAPK and inhibited cell proliferation and migration in hepatocellular carcinoma. Onco Targets Ther 9: 325-333, 2016.
42. Milovanovic P, Rakocevic Z, Djonic D, Zivkovic V, Hahn M, Nikolic S, Amling M Busse B and Djuric M: Nano-structural, compositional and micro-architectural signs of cortical bone fragility at the superolateral femoral neck in elderly hip fracture patients vs. healthy aged controls. Exp Gerontol 55: 19-28, 2014.
43. Wilson JM, Kunnimalaiyaan S, Gamblin TC and Kunnimalaiyaan M: MK2206 inhibits hepatocellular carcinoma cellular proliferation via induction of apoptosis and cell cycle arrest. J Surg Res 191: 280-285, 2014.
44. Jiang W, Huang H, Ding L, Zhu P, Saiyin H, Ji G, Zuo J, Han D, Pan Y, Ding D, *et al*: Regulation of cell cycle of hepatocellular carcinoma by NF90 through modulation of cyclin E1 mRNA stability. Oncogene 34: 4460-4470, 2015.
45. Liu YS, Tsai YL, Yeh YL, Chung LC, Wen SY, Kuo CH, Lin YM, Padma VV, Kumar VB and Huang CY: Cell cycle regulation in the estrogen receptor beta (ESR2)-overexpressing hep3b hepatocellular carcinoma cell line. Chin J Physiol 58: 134-140, 2015.
46. Meng X, Franklin DA, Dong J and Zhang Y: MDM2-p53 pathway in hepatocellular carcinoma. Cancer Res 74: 7161-7167, 2014.
47. Sun J, Wang B, Liu Y, Zhang L, Ma A, Yang Z, Ji Y and Liu Y: Transcription factor KLF9 suppresses the growth of hepatocellular carcinoma cells in vivo and positively regulates p53 expression. Cancer Lett 355: 25-33, 2014.
48. Li X, Yu J, Brock MV, Tao Q, Herman JG, Liang P and Guo M: Epigenetic silencing of BCL6B inactivates p53 signaling and causes human hepatocellular carcinoma cell resist to 5-FU. Oncotarget 6: 11547-11560, 2015.
49. Wang P, Cui J, Wen J, Guo Y, Zhang L and Chen X: Cisplatin induces HepG2 cell cycle arrest through targeting specific long noncoding RNAs and the p53 signaling pathway. Oncol Lett 12: 4605-4612, 2016.
50. Baik SH, Lee J, Lee YS, Jang JY and Kim CW: ANT2 shRNA downregulates miR-19a and miR-96 through the PI3K/Akt pathway and suppresses tumor growth in hepatocellular carcinoma cells. Exp Mol Med 48: e222, 2016.
51. Wang TH, Yeh CT, Ho JY, Ng KF and Chen TC: OncomiR miR-96 and miR-182 promote cell proliferation and invasion through targeting ephrinA5 in hepatocellular carcinoma. Mol Carcinog 55: 366-375, 2016.
52. Chen Y, Dong X, Yu D and Wang X: Serum miR-96 is a promising biomarker for hepatocellular carcinoma in patients with chronic hepatitis B virus infection. Int J Clin Exp Med 8: 18462-18468, 2015.
53. Liu Y, Wu C, Wang Y, Wen S, Wang J, Chen Z, He Q and Feng D: MicroRNA-145 inhibits cell proliferation by directly targeting ADAM17 in hepatocellular carcinoma. Oncol Rep 32: 1923-1930, 2014.
54. Ding W, Tan H, Zhao C, Li X, Li Z, Jiang C, Zhang Y and Wang L: MiR-145 suppresses cell proliferation and motility by inhibiting ROCK1 in hepatocellular carcinoma. Tumour Biol 37: 6255-6260, 2016.
55. Ju BL, Chen YB, Zhang WY, Yu CH, Zhu DQ and Jin J: miR-145 regulates chemoresistance in hepatocellular carcinoma via epithelial mesenchymal transition. Cell Mol Biol (Noisy-le-grand) 61: 12-16, 2015.
56. Wang G, Zhu S, Gu Y, Chen Q, Liu X and Fu H: MicroRNA-145 and MicroRNA-133a Inhibited proliferation, migration, and invasion, while promoted apoptosis in hepatocellular carcinoma cells via targeting FSCN1. Dig Dis Sci 60: 3044-3052, 2015.
57. Li J, Fu H, Xu C, Tie Y, Xing R, Zhu J, Qin Y, Sun Z and Zheng X: miR-183 inhibits TGF-beta1-induced apoptosis by downregulation of PDCD4 expression in human hepatocellular carcinoma cells. BMC Cancer 10: 354, 2010.
58. Liang Z, Gao Y, Shi W, Zhai D, Li S, Jing L, Guo H, Liu T, Wang Y and Du Z: Expression and significance of microRNA-183 in hepatocellular carcinoma. ScientificWorldJournal 2013: 381874, 2013.
59. Li ZB, Li ZZ, Li L, Chu HT and Jia M: MiR-21 and miR-183 can simultaneously target SOCS6 and modulate growth and invasion of hepatocellular carcinoma (HCC) cells. Eur Rev Med Pharmacol Sci 19: 3208-3217, 2015.