Screening therapeutic targets of ribavirin in hepatocellular carcinoma

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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 Expression 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 tissues 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 559 DEGs (designated 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.

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 Expression 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 V3.2.10 (http://www.bioconductor.org/packages/3.5/bioc/html/limma.html) (25). According to the criteria: P<0.05 and |log(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(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.

Results

DEGs. A total of 559 DEGs (269 upregulated and 290 downregulated) and 623 DEMs (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 I.

| Gene      | logFC | Average expression | P-value |
|-----------|-------|--------------------|---------|
| NCF2      | 3.636237 | 10.08812 | 4.01x10^{-13} |
| TXNIP     | 3.04897 | 9.867782 | 5.95x10^{-12} |
| PRORSD1P  | 2.527183 | 6.729178 | 4.91x10^{-11} |
| NPPB      | 3.0072 | 12.09607 | 8.26x10^{-11} |
| CYR61     | 2.018572 | 10.7489 | 9.19x10^{-11} |
| PLA2G4C   | 1.93915 | 5.61348 | 9.29x10^{-11} |
| CDKN2B    | 2.282063 | 7.523593 | 9.52x10^{-11} |
| LEAP2     | 2.52559 | 9.937438 | 1.39x10^{-10} |
| DUSP4     | 1.86831 | 8.419143 | 3.18x10^{-10} |
| FLYC1R1-AS1 | 2.01682 | 8.90536 | 4.17x10^{-10} |
| ASH1L-AS1 | 1.803117 | 9.666342 | 4.20x10^{-10} |
| CXCL3     | 1.797497 | 8.092698 | 5.80x10^{-10} |
| GLI2R     | 1.688522 | 9.089448 | 5.91x10^{-10} |
| CYPIA1    | 1.560867 | 11.376 | 7.61x10^{-10} |
| EID2B     | 1.667057 | 8.101158 | 9.18x10^{-10} |
| JUNB      | 1.574027 | 8.65205 | 1.10x10^{-9} |
| BTG2      | 1.498662 | 9.091506 | 1.41x10^{-9} |
| PPL       | 1.94965 | 8.769882 | 2.08x10^{-9} |
| ZNF436-AS1 | 2.570947 | 8.267133 | 2.12x10^{-9} |
| TIGD7     | 1.654807 | 6.61165 | 2.17x10^{-9} |
| LOC284513 | 1.59588 | 6.487373 | 2.40x10^{-9} |
| TUF1      | 1.50993 | 10.31677 | 2.43x10^{-9} |
| CTSE      | 1.439267 | 11.22817 | 2.48x10^{-9} |
| ERP2      | 1.376543 | 10.6632 | 2.51x10^{-9} |
| UCA1      | 1.611053 | 10.73581 | 2.60x10^{-9} |
| HSD3B1    | 1.699597 | 5.372037 | 2.61x10^{-9} |
| GATA6-AS1 | 1.971443 | 9.336978 | 2.99x10^{-9} |
| LOC100134822 | 1.45604 | 7.840603 | 3.38x10^{-9} |
| THUMP3D-AS1 | 1.365701 | 7.84944 | 3.41x10^{-9} |
| GDA       | 1.482742 | 8.376296 | 3.62x10^{-9} |

logFC, log fold-change.

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^{-6} |
| PEA15       | 1.468145 | 8.69977          | 1.04x10^{-6} |
| CENPE       | 2.043658 | 6.056165          | 1.29x10^{-6} |
| C21orf56    | 1.237448 | 5.670084          | 1.54x10^{-6} |
| DBN1        | 1.383559 | 6.53977           | 1.76x10^{-6} |
| GLA         | 1.380323 | 7.172235          | 2.00x10^{-6} |
| DDX39       | 1.17872 | 7.721654          | 2.63x10^{-6} |
| MPV17       | 1.190142 | 9.079669          | 2.67x10^{-6} |
| RFX5        | 1.417855 | 7.558042          | 2.89x10^{-6} |
| TMEM144     | 1.379456 | 5.655336          | 3.59x10^{-6} |
| ASNS        | 2.515006 | 6.603396          | 3.63x10^{-6} |
| SLC38A6     | 1.678498 | 7.591919          | 3.93x10^{-6} |
| GRAMD1A     | 1.013492 | 7.228709          | 4.65x10^{-6} |
| COMMD8      | 1.109678 | 8.353807          | 7.57x10^{-6} |
| YWHAZ       | 1.008572 | 8.92205           | 7.94x10^{-6} |
| PLXNC1      | 1.451381 | 5.772042          | 1.18x10^{-5} |
| PLVP        | 1.001818 | 5.796379          | 1.20x10^{-5} |
| SHCBP1      | 1.327673 | 4.917339          | 1.39x10^{-5} |
| LAMC1       | 1.104793 | 6.905647          | 1.46x10^{-5} |
| ANXA2P2     | 1.89594 | 11.61567          | 1.52x10^{-5} |
| ACTR3       | 1.094921 | 9.606995          | 1.63x10^{-5} |
| CCDC88A     | 1.002649 | 6.195715          | 1.64x10^{-5} |
| E2F3        | 1.093946 | 6.541921          | 1.75x10^{-5} |
| Fam118B     | 1.006248 | 6.9915            | 1.76x10^{-5} |
| ZNF354A     | 1.069078 | 5.918941          | 1.81x10^{-5} |
| RASGEF1A    | 2.021872 | 4.908126          | 1.84x10^{-5} |
| NUP37       | 1.362635 | 6.839194          | 2.01x10^{-5} |
| ESM1        | 1.291572 | 4.762991          | 2.03x10^{-5} |
| KPNA2       | 2.606398 | 9.341649          | 2.13x10^{-5} |
| DCUN1D5     | 1.38889 | 8.463629          | 2.34x10^{-5} |

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^{-10} |
| hsa-mir-106b | 0.32071 | 1.52x10^{-6} |
| hsa-mir-214 | -0.54861 | 7.34x10^{-6} |
| hsa-mir-93 | 0.32346 | 1.73x10^{-5} |
| hsa-mir-10a | -0.51702 | 6.33x10^{-5} |
| hsa-mir-199a-1 | -0.78765 | 6.28x10^{-3} |
| hsa-mir-199a-2 | -0.73035 | 5.57x10^{-11} |
| hsa-mir-301 | 0.5629 | 1.16x10^{-8} |
| hsa-mir-424 | -0.31246 | 1.72x10^{-8} |
| hsa-mir-33 | 0.29308 | 7.92x10^{-26} |
| hsa-mir-324-5p | 0.37868 | 1.01x10^{-21} |
| hsa-mir-25 | 0.20282 | 1.53x10^{-22} |
| hsa-mir-125b | -0.37741 | 1.95x10^{-12} |
| 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.

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
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...
anaphase-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 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.
cells and HCC tissues, and decreased following treatment with ribavirin. Therefore, it is suspected that \textit{NAT2} and \textit{FBX05} 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 (\textit{CCNB1}, \textit{DEPDC1} and \textit{NTN4}) with opposite expression.

### Table VI. Enriched KEGG pathways of DEG-Ribavirin.

| Category       | Pathway name                                           | Gene number | P-value       |
|----------------|--------------------------------------------------------|-------------|---------------|
| KEGG_PATHWAY   | hsa04110: Cell cycle                                   | 15          | 1.19x10^{-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   | hsa0071: Fatty acid metabolism                          | 15          | 1.37x10^{-09} |
| KEGG_PATHWAY   | hsa00280: Valine, leucine and isoleucine degradation    | 14          | 5.58x10^{-08} |
| KEGG_PATHWAY   | hsa04110: Cell cycle                                    | 21          | 1.08x10^{-06} |
| KEGG_PATHWAY   | hsa00830: Retinol metabolism                            | 12          | 3.12x10^{-05} |
| KEGG_PATHWAY   | hsa00380: Tryptophan metabolism                         | 10          | 7.32x10^{-05} |
| KEGG_PATHWAY   | hsa00650: Butanoate metabolism                          | 9           | 3.33x10^{-04} |
| KEGG_PATHWAY   | hsa00250: Alanine, aspartate and glutamate metabolism   | 8           | 4.63x10^{-04} |
| KEGG_PATHWAY   | hsa04114: Oocyte meiosis                                | 15          | 5.67x10^{-04} |
| KEGG_PATHWAY   | hsa00640: Propanoate metabolism                         | 8           | 5.69x10^{-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: -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.
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

El-Serag HB: Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology 142: 1264-1273.e1, 2012.

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.

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.

Lopez PM. Villameña A and Llovet JM: Systematic review: Exendid90-based management of hepatocellular carcinoma-an updated analysis of randomized controlled trials. Aliment Pharmacol Ther 23: 1535-1547, 2006.

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.

Ji Z, Meng G, Huang D, Yue X and Wang B: NMFFBS: A NMF-based feature selection method in identifying pivotal clinical symptoms of hepatocellular carcinoma. Comput Math Methods Med 2015: 846942, 2015.

Paul SB, Shalimar, Sreenivas V, Gamanagatti SR, Sharma H, Dhamija E and Acharya SK: Incidence and risk factors of hepatocellular carcinoma (HCC) -an investigation in a department of surgical oncology study. Support Care Cancer 22: 919-926, 2014.

Diboun I, Vernisch L, Ongenae CA and Koltzenburg M: Microarray analysis after RNA amplification can detect pronounced differences in gene expression using limma. BMC Genomics 7: 252, 2006.

Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al: DAVID: Database for annotation, visualization, and integrated discovery. Genome Biol 4: P3, 2003.

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.

Thomas E, Feld JJ, Liu Q, Ho C, 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.

Burchard J, Zhang C, Liu AM, Poon RT, Lee NP, Wang LF, Shan PC, Lam BY, Ferguson MD, Tokiwa G, et al: miRNA-122 as a regulator of mitochondrial metabolic gene network in hepato cellular carcinoma. Mol Syst Biol 6: 402, 2010.

Liu AM, Yao TJ, Wang W, Wang LF, 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.

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. Bioinformatics 21: 249-254, 2003.

Liu CJ, Chu YT, Shau WY, Kuo RN, Chen PJ and Lai MS: Risk factors for hepatocellular carcinoma in NAT2-slow acetylators and CYP2D6- rapid metabolizers. Pharmacogenomics 7: 252, 2006.

Dennis G Jr, Sherman B, Hosack DA, Yang J, Gao W, Lane HC, et al: DAVID: Database for annotation, visualization, and integrated discovery. Genome Biol 4: P3, 2003.

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.

Dennis G Jr, Sherman B, Hosack DA, Yang J, Gao W, Lane HC, et al: DAVID: Database for annotation, visualization, and integrated discovery. Genome Biol 4: P3, 2003.

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.
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-Acetyltransferase10-2, glutathione S-transferase M1 and T1 genetic polymorphisms, cigarette smoking and hepatocellular carcinoma: A case-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: Large-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: Em1 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 anaphase10-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 co-chaperone 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. Oncol Targets Ther 9: 325-333, 2016.

42. Milovanovic P, Rakocvic Z, Djonc 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, Kannimalaiyaan S, Gamblin TC and Kannimalaiyaan 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: 4461-4470, 2015.

45. Liu YS, Tai 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 (ER2)-overexpressing hep3b hepatocellular carcinoma cell line. Chin J Physiol 58: 134-140, 2015.

46. Wang 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 YJ, 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, Wang X, Sun CK, Chua MS and So S: Suppressing the 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. Tumour Biol 37: 6255-6260, 2016.

58. Blum HE: Hepatocellular carcinoma: Susceptibility markers. IARC Sci Publ 154: 241-244, 2001.