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How Hepatitis C Virus Leads to Hepatocellular Carcinoma: A Network-Based Study

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Abstract

Background: Hepatitis C virus (HCV) has been known as a major cause of hepatocellular carcinoma (HCC) worldwide. However, the distinct molecular mechanisms underlying the effects of HCV proteins on the HCC progression have remained unclear.
Objectives: In the present study, we studied the possible role of HCV in the HCC initiation and invasion using topological analysis of protein-protein interaction (PPI) networks.
Materials and Methods: After analysis with GEO2R, a PPI network of differentially expressed genes (DEGs) was constructed for both chronic HCV and HCC samples. The STRING and GeneMANIA databases were used to determine the putative interactions between DEGs. In parallel, the functional annotation of DEGs was performed using g: Profiler web tool. The topological analysis and network visualization was carried outperformed using Cytoscape software and the top hub genes were identified. We determined the hub genes-related miRNAs using miRTarBase server and reconstructed a miRNA-Hubgene network.
Results: Based on the topological analysis of miRNA-Hubgene network, we identified the key hub miRNAs. In order to identify the most important common sub-network, we aligned two PPI networks using NETAL tool. The *c-Jun* gene was identified as the most important hub gene in both HCV and HCC networks. Furthermore, the *hsa-miR-34a*, *hsa-miR-155*, *hsa-miR-24*, *hsa-miR-744* and *hsa-miR-92a* were recognized as the most important hub miRNAs with positive correlation in the chronic HCV and HCC samples. Functional annotation of differentially expressed miRNAs (DEMs) using the tool for annotations of human miRNAs (TAM) revealed that there is a considerable overlap between *miRNA* gene expression profiles of HCV-infected and HCC cells.
Conclusions: Our results revealed the possible crucial genes and miRNAs involved in the initiation and progression of HCC cells infected with HCV.

Keywords: HCV, HCC, Topological Analysis, PPI Networks, miRNA

1. Background

Hepatitis C virus (HCV) is the most common cause of chronic liver disease and also an important factor in liver transplantation (CDC, www.cdc.gov/hepatitis/hcv/hcvfaq.htm). The world health organization (WHO) has estimated that 3% of the world's population has been infected with hepatitis C virus (HCV) and currently more than 170 million carry chronic HCV (WHO, www.who.int). HCV is considered the cause of approximately one-third of HCC cases in the USA. According to various reports, it is estimated that the risk of development of HCC when liver cirrhosis has progressed is 0 to 3% per year. The risk of HCC in chronic infections of HCV is highly associated with fibrosis stage. The incidence of HCC is relatively high in patients with cirrhosis (1% - 7% per year) (1, 2).

Experimental observations have shown that HCV viral proteins interact with cell proliferation and survival pathways, which lead to the increased risk of HCC. The overexpression of HCV protein enhances cell proliferation, transformation, and tumor development in mice, which suggests that viral proteins lead to the activation of oncogenic pathways (3). Several studies have shown that core protein can participate in liver cancer caused by HCV. It has been shown that the core protein expression can modulate cellular mRNA transcription through the interaction with hnRNP K (4). It also has been shown that the core protein can affect p53 protein through various mechanisms such

as physical connection (5, 6). Studies have suggested that non-structural HCV proteins may also play a role in carcinogenesis (7). In parallel with this hypothesis, it has been reported that the expression of three HCV genes, including *NS3*, *NS5A* and *NS5B* can affect cell proliferation. *NS3* and *NS5A* proteins can inhibit transcription of *p21* (8-10) and *NS5A* protein impairs the cell cycle pathway by targeting *cdk1/2*-cyclin complex (11). *NS5A* protein also activates the Wnt pathway and stimulates the β-catenin-responsive transcription by activating the phosphoinositide 3-kinase (12). Finally, expression of non-structural *NS5B* protein, by targeting proteasomal degradation of pRB, leads to up-regulation of *E2F*-responsive promoter and induction of cell entry into the S phase of the cell cycle (13).

Although the molecular mechanisms of HCV-induced HCC remain to be discovered, studies have suggested that HCV is the important factor in providing an appropriate microenvironment for progression and development of cancer (14). In addition, gene expression alterations of chronic liver disease and molecular mechanisms involved in the HCV-induced HCC are not well understood.

2. Objectives

Nowadays, the network-based analysis of high-throughput gene expression profiles is emerging as a reliable tool in the perception of biological processes. In the present study, we studied the possible role of HCV in HCC initiation and invasion using topological analysis of PPI networks.

3. Materials and Methods

3.1. Microarray Data

The gene expression profile GSE38226 was obtained from the public functional genomics data repository gene expression omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo/). There are two Illumina platforms in this transcription profile, including GPL6947 and GPL8179 used as mRNA and miRNA expression beadchips, respectively. Before the mRNA and miRNA expression profiling, the liver biopsies were collected from chronic HCV patients (24 samples), patients with hepatocellular carcinoma (12 samples) and normal subjects (8 samples). The samples were used to identify the differentially expressed genes (DEGs) and differentially expressed miRNAs (DEMs) using GEO2R (15).

3.2. Functional Annotation of DEGs and DEMs

Gene ontology (GO) annotation of all DEGs was performed using g: Profiler public web server (http://biit.cs.ut.ee/gprofiler/). g: Profiler is a powerful web tool for characterizing and manipulating the gene lists obtained from analysis of the high-throughput genomic data. The overrepresented GO terms with significant P value filtered from the primary results.

To determine functional categories of DEMs obtained from the previous step, the list of DEMs of both chronic HCV and HCC expression profiles were introduced to the TAM tool (16). The list of DEMs was functionally annotated and summarized according to the integrated miRNA categorical data. The important functional categories were chosen for further analysis. There was a considerable overlap between the enrichment results of chronic HCV and HCC in the given DEMs lists.

3.3. Protein-Protein Interaction (PPI) Network Construction

Based on the STRING (a protein-protein interaction database) (17) and GenMANIA (a web service used for prediction of gene function) regulation databases, we constructed and visualized the corresponding PPI networks using Cytoscape software (18). We considered directed interactions as well as important undirected interactions such as genetic interactions for building PPI networks. The number of nodes-edges in the PPI networks constructed from chronic HCV and HCC gene expression profiles were 171 - 1090 and 175 - 1161, respectively.

3.4. Topological Analysis of PPI Networks

Topological network analysis was performed for PPI networks and the most important hub genes were identified. The network analyzer plugin of cytoscape was used to analyze topological properties of the networks. Among nine evaluated methods, degree, betweenness centrality and closeness centrality had better outcomes and used for topological analysis of the PPI networks. Degree measure refers to the number of neighbors (edges) of a node and the nodes with higher degree centrality are considered biologically important hub genes within biological networks. Betweenness centrality is another centrality measure that represents the number of times each node is visited during traversing all shortest paths and is computed as follows:

$$C_b(n) = \Sigma_{s \neq n \neq t} \left(\frac{\sigma_{st}(n)}{\sigma_{st}} \right) \quad (1)$$

The s and t are nodes in the network different from n, σ_{st} indicates the number of shortest paths from s to t and $\sigma_{st}(n)$ is the number of shortest paths from s to t that n lies on.

The closeness centrality of a node is a measure of how fast information spreads from a given node to other reachable nodes in the given network. This measure is computed as follows:

$$C_c(n) = \frac{1}{\text{avg}(L(n,m))} \quad (2)$$

Where L (n, m) is the length of the shortest path between two n and m nodes. The closeness centrality of each node is a number between 0 and 1.

We selected the top 50 genes according to the ranked results of three mentioned methods. Furthermore, the putative miRNAs regulating hub genes were predicted *in silico* using mirTarBase server (19) and a new miRNA-Hub-gene network was reconstructed for both chronic HCV

and HCC gene expression profiles. Topological analysis of the new miRNA-Hubgene networks was also performed using the degree measure to identify the hub miRNAs in each network. Interestingly, most hub miRNAs had been determined as DEMs after miRNA expression analysis of chronic HCV and HCC samples with GEO2R.

3.5. PPI Networks Alignment

A network alignment allows us to find the conserved functional complexes between two or more given networks. The alignment between chronic HCV and HCC networks was performed by NETAL tool (20). The NETAL is a new method for global alignment of PPI networks based on both biological and topological information of input networks. This method is highly capable of finding the conserved functional complexes and evolutionary relationships. The edge correctness (EC) measure was considered an important parameter to determine topological similarity between chronic HCV and HCC derived PPI networks. EC is the percentage of interactions aligned between the given networks. After comparative analysis of chronic HCV and HCC networks, the most important common sub-network was determined and visualized.

4. Results

4.1. Functional Annotation Analysis

Analysis of the functional annotation of chronic HCV and HCC related DEGs specifically indicated that most genes, in both chronic HCV and HCC related DEGs, are involved in important cellular processes such as cell signaling, cell proliferation, cell death, cell migration, etc. (Table 1). These functional categories are obviously involved in the cancer progression and invasion.

Furthermore, we enriched the list of DEMs obtained from miRNA expression analysis of both HCC and chronic HCV samples. Interestingly, there was a considerable overlap between the functional categories of chronic HCV and HCC related DEMs (Figure 1). Similarly to DEGs, a high number of miRNAs predicted as regulators of several cancer-related pathways such as cell cycle, cell proliferation, cell death, apoptosis, epithelial-mesenchymal transition and angiogenesis. We found that the four important onco-miRNA (*hsa-miR-24*, *hsa-miR-92a*, *hsa-miR-194* and *hsa-miR-191*) were up-regulated in both chronic HCV and HCC samples compared to the control liver cells. Furthermore, several miRNA tumor suppressors, including *hsa-miR-181b*, *hsa-miR-451*, *hsa-miR-34a*, *hsa-miR-34b* and *hsa-miR-34c* were significantly down-regulated in chronic HCV and HCC samples compared to the control samples.

4.2. Topological Analysis of PPI Networks

The PPI networks for DEGs resulted from analysis of chronic HCV and HCC gene expression profiles were constructed with 171 - 1090 and 175 - 1161 nodes-edges, respectively. We used the degree, betweenness centrality and closeness centrality measures to analyze topology of resulted PPI networks. The top 50 hub genes of these three measures were selected for further analysis (Tables 2 and 3). After filtering and removing duplicate values, we obtained 61 and 64 unique hub genes from the topological analysis of chronic HCV and HCC related PPIs, respectively. There were 32 common hub genes between chronic HCV and HCC unique hub genes. Interestingly, the c-Jun gene was identified as the most important hub gene in both chronic HCV and HCC related PPI networks. In parallel,

Table 1. Overrepresented gProfiler Terms in the DEGs From HCC and Chronic HCV

DEGs Source	Number of Genes	P Value
HCC		
Localization	85	1.58e - 03
Signaling	87	2.68e - 02
Cell proliferation	38	2.39e - 02
Cell death	37	3.14e - 02
Cell migration	36	5.18e - 06
Extracellular matrix	75	3.15e - 04
Chronic HCV		
Signaling	101	1.66e - 07
Localization	81	2.12e - 02
Cell adhesion	56	3.19e - 17
Immune response	59	3.68e - 08
Cell death	40	7.57e - 03
Angiogenesis	21	1.05e- 06

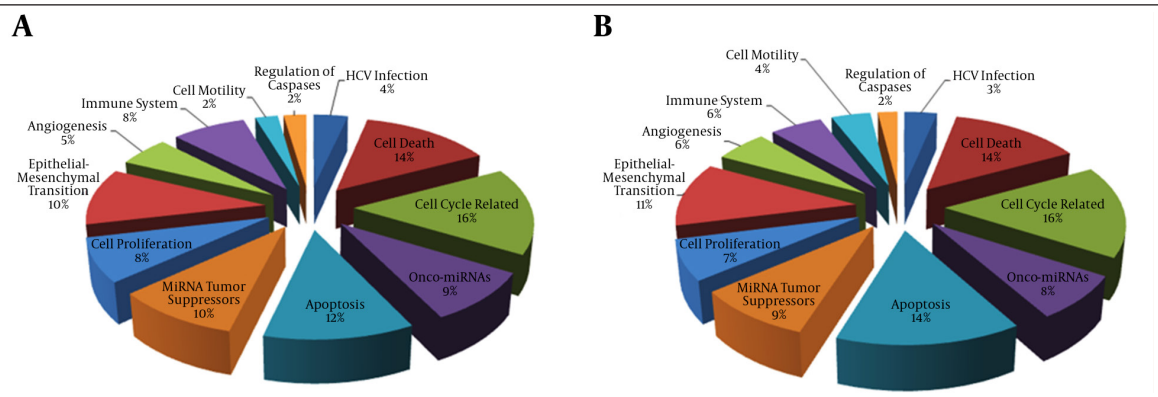


Figure 1. Functional Enrichment Analysis of DEMs Resulted From miRNA Expression Analysis of Chronic HCV and HCC Samples

validated corresponding miRNAs for each hub gene were determined and a new miRNAs-Hubgenes network was reconstructed with 217 and 319 nodes and edges, respectively. Topological analysis of miRNAs-Hubgenes regulatory network was performed and the hub miRNAs were identified and visualized along with their putative targets (Figure 2). We compared the hub miRNAs with DEMs obtained from the miRNA expression profile of chronic HCV and HCC samples. The comparison indicated a positive correlation between chronic HCV and HCC resulted DEMs. Two hub miRNAs, including *hsa-miR-34a* and *hsa-miR-155* were significantly down-regulated in both chronic HCV and HCC samples compared to normal liver samples. Moreover, three other hub miRNAs (*hsa-miR-24*, *hsa-miR-744* and *hsa-miR-92a*) were up-regulated in chronic HCV and HCC samples compared to the control samples.

Table 2. The Top 50 Hub Genes Obtained From Analysis of the Chronic HCV Network

Betweenness	Value	Degree	Value	Closeness	Value
JUN	0.088456	JUN	60	JUN	0.563107
KCNMA1	0.045263	KCNMA1	38	KCNMA1	0.537037
SLC1A1	0.044697	COL4A1	36	SLC1A1	0.52568
BCL2	0.035565	SLC1A1	35	LEF1	0.517857
COL4A1	0.035263	LEF1	33	COL4A1	0.514793
PODXL	0.031336	BCL2	32	ACACB	0.513274
BIRC3	0.030732	VEGFC	31	ENAH	0.511765
VEGFC	0.029737	COL1A2	31	BCL2	0.508772
WNT4	0.029547	BIRC3	30	VEGFC	0.507289
ENAH	0.02707	ENAH	30	COL1A2	0.501441
POLQ	0.026244	SOX9	29	PID1	0.501441
ACACB	0.025908	COL5A1	29	PODXL	0.5
LEF1	0.025767	COL15A1	29	WNT4	0.5
CELF4	0.024233	PODXL	28	BIRC3	0.498567
FANCC	0.022376	WNT4	28	COL15A1	0.497143
SLCO3A1	0.022328	ACACB	28	CELF4	0.494318
TSPAN8	0.021671	SLCO3A1	28	SLCO3A1	0.492918
SMOC2	0.020669	MYOF	28	CD34	0.492918
SOX9	0.020065	TSPAN8	27	BCAS4	0.491525
TMEM200A	0.019163	SOX4	26	MYOF	0.490141
SOX4	0.01913	BCAS4	26	TSPAN8	0.490141
STK39	0.018437	THBS2	26	AGTRAP	0.490141
COL5A1	0.017963	CD44	26	BRCA1	0.488764
CBLN1	0.017321	CELF4	25	KCNN2	0.487395
ADCY1	0.016641	PID1	25	SOX9	0.486034
OSBPL3	0.016441	COL4A2	25	SOX4	0.486034
BCAS4	0.01626	ADRA1A	24	SPP1	0.486034
MYOF	0.015722	CD34	24	STK39	0.483333
COL1A2	0.014797	STK39	23	COL5A1	0.481994
THBS2	0.014385	BRCA1	23	PDGFRB	0.481994
CCL20	0.014217	SPP1	23	FRZB	0.481994
TGFBI	0.014096	CHRM3	23	CHRM3	0.480663
FRZB	0.014042	KCNN2	23	SMOC2	0.480663
COL15A1	0.014024	POLQ	22	CBLN1	0.480663
CD44	0.013973	FANCC	22	ERBB2	0.479339
FST	0.013728	SMOC2	22	THBS2	0.476712
STAG3	0.01337	TMEM200A	22	ADRA1A	0.476712
S100A13	0.013157	CBLN1	22	POLQ	0.476712
ACSL1	0.012823	TGFBI	22	VWF	0.476712
DACT2	0.012597	ST3GAL6	22	PARM1	0.476712
BRCA1	0.01237	COL1A1	22	NOS1AP	0.476712
RRM2	0.012267	OSBPL3	21	ST3GAL6	0.47541
ADRA1A	0.012258	PDGFRB	21	COL1A1	0.47541
PLVAP	0.011888	B3GALNT1	20	CMIP	0.47541
SPP1	0.011585	ERBB2	20	TGFBI	0.472826
B3GALNT1	0.011486	AGTRAP	20	TMEM200A	0.471545
SFRP5	0.011395	ADCY1	19	ASPH	0.47027
CHRM3	0.011097	STAG3	19	CD44	0.469003
KCNN2	0.01109	DCDC2	19	SERPINE2	0.469003
PID1	0.011078	VWF	19	COL4A2	0.467742

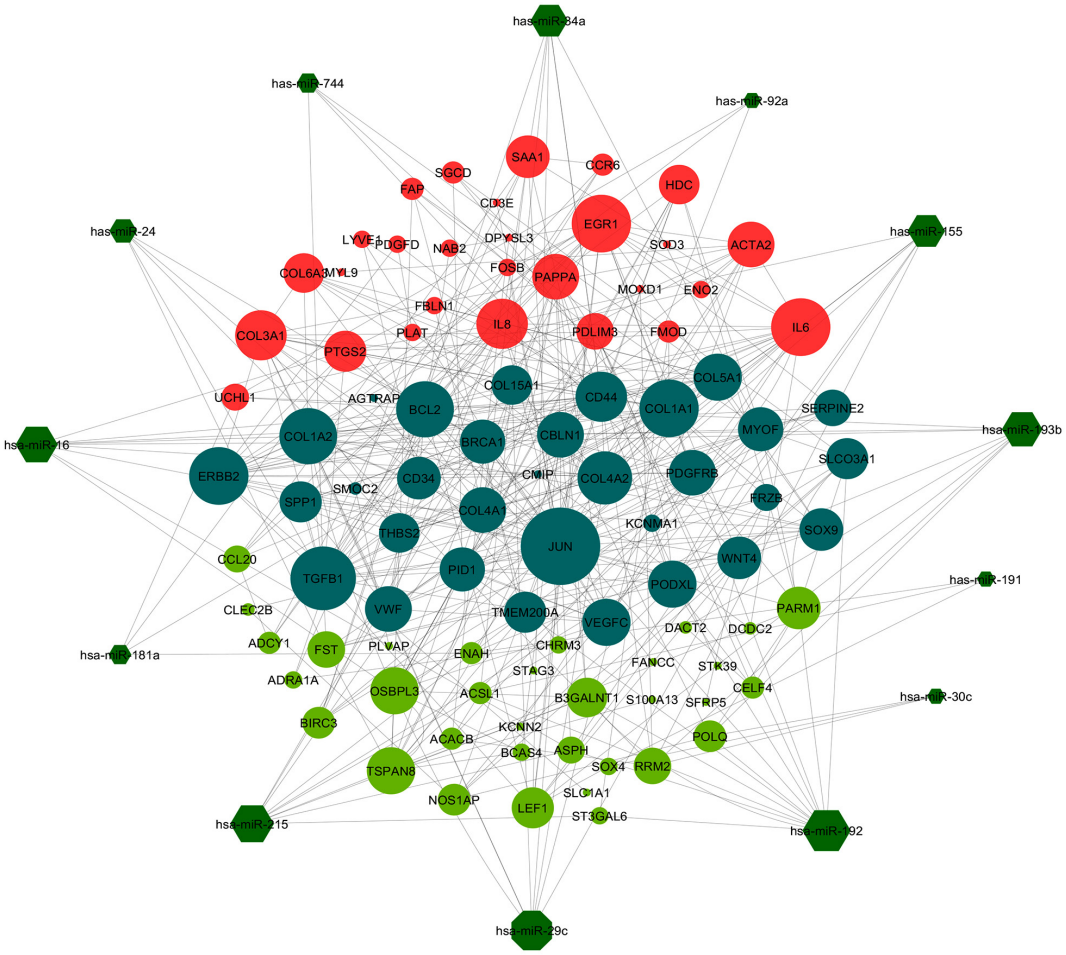
Table 3. The Top 50 Hub Genes Obtained From Analysis of the HCC Network

Betweenness	Value	Degree	Value	Closeness	Value
JUN	0.152054	JUN	79	JUN	0.602837
PAPPA	0.064493	BCL2	39	BCL2	0.532915
BCL2	0.04056	KCNMA1	36	COL1A2	0.519878
KCNMA1	0.040488	COL1A2	36	PAPPA	0.519878
VEGFC	0.038859	PAPPA	35	VEGFC	0.519878
PDGFD	0.037164	SOX9	33	KCNMA1	0.516717
COL4A1	0.033355	COL4A1	32	COL4A1	0.515152
ERBB2	0.033017	CD44	32	ERBB2	0.515152
COL1A2	0.031187	EGR1	32	SOX9	0.505952
SOX9	0.029953	VEGFC	31	EGR1	0.505952
IL8	0.028423	ERBB2	29	FMOD	0.504451
CCR6	0.02778	IL8	28	SOD3	0.501475
FMOD	0.024919	FMOD	27	PODXL	0.5
PODXL	0.023075	PODXL	27	CD34	0.5
CD44	0.022533	WNT4	27	PDGFRB	0.5
EGR1	0.021941	IL6	27	CD44	0.498534
MOXD1	0.02144	COL5A1	27	WNT4	0.498534
SPP1	0.020341	MOXD1	26	SPP1	0.498534
CD34	0.019815	CD34	26	IL8	0.497076
PDLIM3	0.018409	SPP1	25	COL1A1	0.497076
WNT4	0.017109	COL4A2	25	MOXD1	0.494186
SLCO3A1	0.017079	COL15A1	25	IL6	0.492754
IL6	0.016092	COL1A1	24	UCHL1	0.489914
TGFBI	0.015425	SLCO3A1	23	FRZB	0.489914
LYVE1	0.015167	SOD3	23	VWF	0.487106
HDC	0.014886	PDGFRB	23	COL5A1	0.48433
SOD3	0.014787	COL6A3	23	SLCO3A1	0.482955
MYOF	0.014711	PDGFD	22	AGTRAP	0.481586
PTGS2	0.014547	MYOF	22	PID1	0.478873
SMOC2	0.014195	TGFBI	21	PTGS2	0.478873
COL4A2	0.013321	SMOC2	21	COL6A3	0.477528
UCHL1	0.013228	UCHL1	21	COL3A1	0.477528
COL1A1	0.013075	VWF	21	COL4A2	0.47619
VWF	0.01304	PID1	21	TGFBI	0.47619
COL5A1	0.013023	FRZB	21	SMOC2	0.47619
CBLN1	0.012941	HDC	20	SGCD	0.47619
PID1	0.012849	PTGS2	20	NAB2	0.47619
MYL9	0.012038	CCR6	19	BRCA1	0.47486
FAP	0.011566	SGCD	19	CBLN1	0.47486
FRZB	0.011414	NAB2	19	COL15A1	0.473538
ENO2	0.010941	COL3A1	19	MYOF	0.473538
TMEM200A	0.01088	TMEM200A	18	HDC	0.472222
SGCD	0.009918	BRCA1	18	CMIP	0.470914
CD3E	0.009637	FOSB	17	ACTA2	0.469613
PDGFRB	0.009418	THBS2	17	PDGFD	0.467033
COL15A1	0.00936	PDLIM3	16	CCR6	0.465753
PLAT	0.009294	CBLN1	16	DPYSL3	0.465753
FBLN1	0.009258	FAP	16	FOSB	0.464481
BRCA1	0.008361	CD3E	16	SERPINE2	0.464481
CLEC2B	0.008261	SAA1	16	PLAT	0.464481

4.3. PPI Networks Alignment

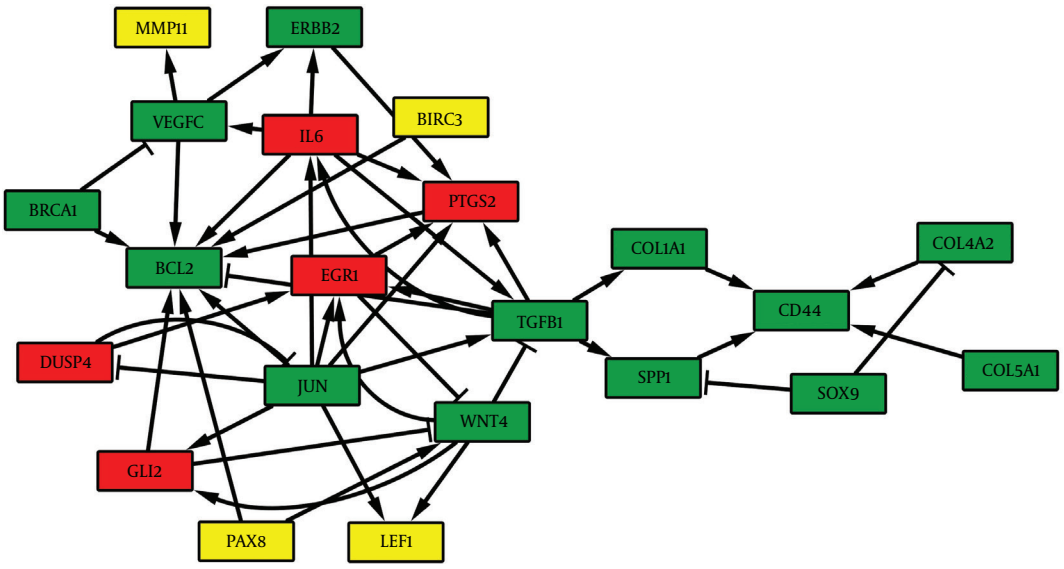
After networks alignment, several sub-networks were identified as common complexes between chronic HCV and HCC networks. The EC measure was equal to 0.45 indicating approximately 45% of topological similarity between the given networks. The connected component between the two PPI networks had 156 nodes and 471 edges. We selected a 22-node sub-network as the most important functional complex, which consists of 13 common genes with positive correlation (Figure 3). The functional annotation analysis demonstrated that this sub-network is actively involved in regulation of cell proliferation, cell migration and apoptosis.

Figure 2. The Regulatory Network of Hub miRNAs (Dark Green Hexagons) Along With Hub Genes of Chronic HCV (Red Circles) and HCC (Pale Green Circles) PPI Networks



Nodes with higher degrees are shown in the bigger circles. The common hub genes between chronic HCV and HCC are shown with dark cyan circles.

Figure 3. The Most Important Functional Sub-Network Obtained From Two PPI Network Alignments



The common genes between chronic HCV and HCC networks are shown in the green color boxes. The red and yellow boxes represent chronic HCV and HCC specific genes, respectively.

5. Discussion

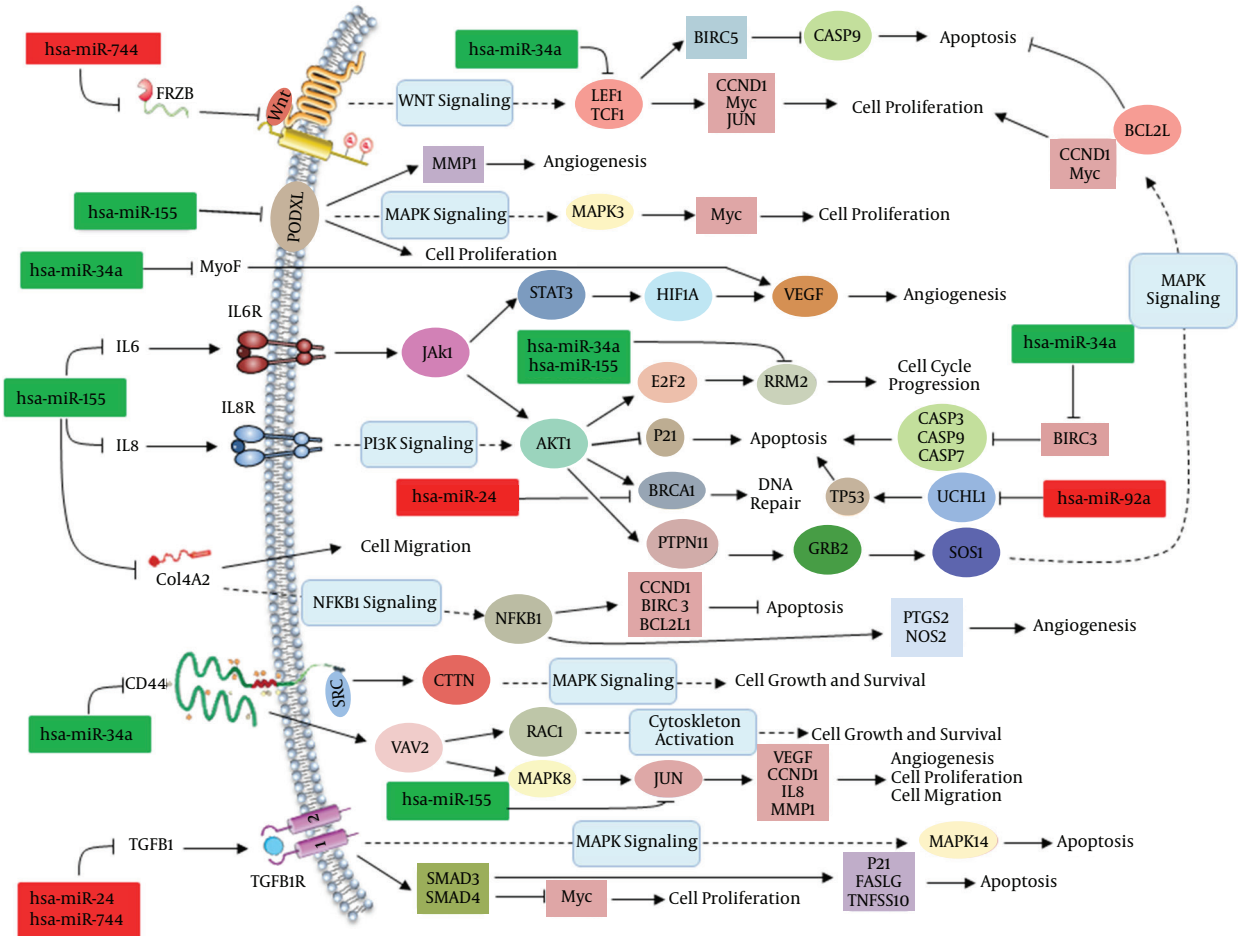
Strong epidemiological evidence links hepatitis C infection with hepatocellular carcinoma (3). Liver cancer usually develops decades after HCV infection and liver cirrhosis. However, the molecular mechanisms involved in cancer development have not been fully understood. HCV is classified as a positive-strand RNA virus that replicates in the cytoplasm and does not integrate into the host DNA; however, its carcinogenic features are remarkable. It is possible that indirect mechanisms such as chronic liver inflammation, oxidative damages, presence of cirrhosis and DNA damage are involved in cancer development (21, 22). Cirrhosis as the result of fibrosis due to hepatitis C infection is the indication of preneoplastic stage in development of liver cancer, which can cause rapid proliferation of dysplastic liver cells (21). Studies have shown that HCV proteins, especially core protein, are inherently oncogenic and their expression in the cell can be involved in the development of HCC directly and in the absence of immune responses or cirrhosis. Several HCV proteins can interact with host cell proteins and induce cell proliferation. Evidence has shown that replication of HCV replicon RNAs is strongly dependent on cell proliferation (23), while the HCV RNA synthesis is stimulated in the S phase of the cell cycle (24). Hepatocytes usually have low proliferation rate and it seems that some viral proteins expressed during HCV infection trigger cell division and are involved in carcinogenic effects of chronic HCV infection (22, 25).

A positive correlation between chronic HCV infection and HCC progression has been frequently reported. However, the main mechanisms underlying pathogenesis of HCV-induced HCC has remained unclear. We reported here molecular mechanisms regulated by HCV, which seem to be involved in HCC progression and invasion. We found that the *c-Jun* gene is the most important hub gene obtained from topological analysis of both HCV and HCC networks. The *c-Jun* has a key role in enhanced cell proliferation of cancer cells by regulating the expression of *CCND1* and *Cdc2* genes (26). In addition, *c- Jun* can prevent *TNFα*-mediated apoptosis through cooperation with *NFKB1*. Erhadt and colleagues reported that HCV can protect various cells from *TNFα*-induced apoptosis via activating *NFKB* signaling pathway (27). Taken together, we suggest that the role of HCV in the inhibition of apoptosis is mediated by c- Jun function. Two proto-oncogene, *BCL2* and *ERBB2*, were identified as the common hub genes of HCV and HCC-derived PPI networks. The *BCL2* overexpressing cells tend to have a prolonged survival and reduced apoptosis in a variety of cancer cells. This gene is highly capable of suppressing apoptosis by either inhibiting the release of cytochrome c from the mitochondria or blocking the function of *APAF1* (28). It is now well established that HCV triggers cancer cell apoptosis and survival through up-regulation of *BCL2* expression, resulting in reduced *CASP3* activity. The nonstructural protein 4B (*NS4B*) is the key gene responsible for stimulating *BCL2* gene through inhibition of the function of *SOCS3* gene (29). *ERBB2* is a protein tyrosine kinase that can bind to other growth factor receptors to form a heterodimer, stabilizing ligand-receptor complex and enhancing several downstream signaling pathways such as mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K/Akt1) (10, 30). This gene was identified as an important hub gene in both chronic HCV and HCC networks. The statistically significant correlation between *NS3*, an HCV serine protease, and *ERBB2* has been reported in HCC cases (31). VEGFC is an important gene involved in promoting angiogenesis. This hub gene was up-regulated in HCV and HCC samples compared to normal liver cells. It is now widely accepted that HCV is of central importance for vascular development and angiogenesis through enhancing the expression of *VEGFC* (32).

Extracellular matrix proteins play a pivotal role in tumor progression and their alterations are usually associated with an aggressive phenotype of various cancer types. Our results revealed that expression of *COL4A2*, *COL1A1* and *COL5A1* hub genes were significantly altered in the HCV-infected and HCC cells compared to the normal cell samples. These genes are necessary for spreading HCC cells through remodeling of extracellular matrix (33). Recently, it has been determined that HCV infection can lead to an increased invasive phenotype by regulating the matrix metalloproteinases (MMPs), a family of extracellular matrix components (34). However, the role of HCV in regulation of collagen genes is poorly understood.

Amongst non-coding RNAs, the role of miRNAs in carcinogenesis has been extensively investigated. Varnholt and colleagues examined the miRNA gene expression profile of HCV-infected HCC cells and identified several important miRNAs that seem to be involved in the pathogenesis of HCV-associated HCC (26). We found that the miRNA gene expression profile of HCV-infected cells is clearly similar to HCC cells. The *hsa-miR-155* and *hsa-miR-34a* were down-regulated in both HCV-infected and HCC cells compared to the control samples. The HCV core and *NS5A* proteins have been implicated in pathogenesis of HCC through induction of Wnt signaling pathway (28). HCV-associated activation of Wnt signaling pathway may be mediated by expression alterations of *hsa-miR-34a* and *has-miR-744*. Additionally, the HCV can actively induce cell proliferation and MAPK signaling pathway in vitro (27). However, the distinct mechanism defining the effect of HCV on the cell proliferation has remained unknown. Here, we show that HCV can positively impair cell cycle progression through inhibition of hsa-miR-34a. The expression of two cell proliferation-related genes, RRM2 and BIRC3, is regulated by *hsa-miR-34a* (Figure 4). This miRNA can activate the MAPK signaling pathway.

Figure 4. The Hub miRNAs Obtained From Topological Analysis of miRNA-Hubgene Network Along With Their Putative Targets



Up-regulated and down-regulated hub miRNAs with positive correlation in both HCV-infected and HCC cells are shown in red and green boxes, respectively.

However, activation of MAPK, AKT1, NFKB1 and JAK-STAT signaling pathways is also mediated through down-regulation of *hsa-miR-155*. This hub miRNA was significantly down-regulated in both HCV-infected and HCC cells compared to normal cells. However, functional study of *hsa-miR-155* targets revealed that down-regulation of this miRNA can induce cell proliferation and angiogenesis and inhibit apoptosis through activating aforementioned signaling pathways (Figure 4). The *hsa-miR-24* acts as an anti-apoptotic miRNA by repressing the expression of *Bim* gene (35). We found that this hub miRNA was up-regulated in HCV-infected and HCC samples, suggesting its possible effect on the progression of HCC cells. Furthermore, the function of tumor suppressor *BRCA1* is considerably modulated by *hsa-miR-24*. The *hsa-miR-92a* is a key regulator of angiogenesis and apoptosis. It has been shown that overexpression of *hsa-miR-744* can moderately induce tube formation and reduce apoptosis in vitro (36).

5.1. Conclusion

This study indicated that characteristics of invasive phenotype HCC cells are associated with differential expressions of several genes and miRNAs. The graph-based information may give us a new insight into molecular mechanisms underlying the pathogenesis of HCV-induced HCC.

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Footnote

Authors' Contribution: Vahdat Poortahmasebi: literature search, writing and reviewing and bioinformatic analysis; Mansour Poorebrahim: study concept

and design, main writing part and bioinformatic analysis; Saeideh Najafi: data collection and writing; Seyed Mohammad Jazayeri, Seyed Moayed Alavian, and Seyed Shahriar Arab: participated in the design of study and reviewed manuscript; Saeid Ghavami: critical revision of the manuscript for important intellectual content; Seyed Ehsan Alavian: Adel Rezaei Moghadam, and Mehdi Amiri: contributed equally to this paper. All authors read and approved the final manuscript.

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