Bioinformatics analysis of key genes and pathways for hepatocellular carcinoma transformed from cirrhosis

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Abstract

Objective: We aimed to identify some pivotal genes and pathways for hepatocellular carcinoma (HCC) transformation from cirrhosis and explore potential targets for treatment of the disease.

Methods: The GSE17548 microarray data were downloaded from Gene Expression Omnibus database, and 37 samples (20 cirrhosis and 17 HCC samples) were used for analysis. The differentially expressed genes (DEGs) in HCC tissues were compared with those in cirrhosis tissues and analyzed using the limma package. Gene ontology-biological process and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analyses were performed using ClueGO and CluePedia tool kits, and the key KEGG pathway was analyzed using the R package pathview. The regulatory factor miRNA of DEGs was extracted from 3 verified miRNAs-target databases using the multiMiR R package. Moreover, a protein-protein interaction (PPI) network was constructed using the Cytoscape software.

Results: DEGs including cyclin-dependent Kinase 1 (CDK1), PDZ-binding kinase (PBK), ribonucleotide reductase M2 (RRM2), and abnormal spindle homolog, and microcephaly-associated drosophila (ASPM) were the hub proteins with higher degrees in the PPI network. The cell cycle pathway (CDK1 enriched) and p53 signaling pathway (CDK1 and RRM2 enriched) were significantly enriched by DEGs.

Conclusion: CDK1, PBK, RRM2, and ASPM may be key genes for HCC transformation from cirrhosis. Furthermore, cell cycle and p53 signaling pathways may play vital mediatory roles; CDK1 may play crucial roles in HCC transformed from cirrhosis via cell cycle and p53 signaling pathways, and RRM2 might be involved in HCC transformed from cirrhosis via the p53 signaling pathway.

Abbreviations: Akt = protein kinase B, ASPM = Abnormal Spindle Homolog, Microcephaly Associated Drosophila, ATIC = 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase, CCND1 = cell cyclin D1, CDK1 = cyclin-dependent kinase 1, DEGs = differentially expressed genes, GEO = Gene Expression Omnibus, GO-BP = gene ontology-biological process, HCC = hepatocellular carcinoma, HLA-DQ = α β heterodimer of type MHC Class II, JAK = Janus kinase, KEGG = Kyoto Encyclopedia of Genes and Genomes, miR-26a = microRNA-26a, mTOR = mammalian target of rapamycin, PBK = PDZ-binding kinase, PI3K = phosphoinositide 3-kinase, RRM2 = ribonucleotide reductase M2, STAT4 = signal transducer and activator of transcription 4.

Keywords: differentially expressed genes, hepatocellular carcinoma, KEGG pathway, miRNA-target genes, protein-protein interaction network

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1. Introduction

Hepatocellular carcinoma (HCC), caused by specific risk factors including aflatoxin exposure and hepatitis virus infections, is the third major cause of cancer-related mortality all over the world.[1,2] The incidence of HCC increases with aging and reaches the peak at age of 70 years.[3] Besides, HCC is 2.4 times more likely to occur in men than in women.[4] It is estimated that >600,000 new HCC cases are diagnosed worldwide every year, and 55% of these cases are in China.[4] Therefore, it is urgent to identify effective treatments for HCC, and additional studies on the molecular mechanisms of HCC may provide new options for the treatment of this disease.

Some reports on the molecular mechanisms of HCC have been published recently. Several studies showed that stathmin could promote cell proliferation and suppress cell apoptosis by regulating the expression of tumor proliferation-related genes, and that it is involved in the development of HCC.[5–8] Furthermore, Jiang et al found that signal transducer and activator of transcription 4 (STAT4) and α β heterodimer of type MHC Class II (HLA-DQ) were 2 genetic susceptibility loci for hepatitis B virus-related HCC.[9] However, AT rich interactive domain 2 (ARID2) is a tumor suppressor gene of HCC,[10] and tumor protein P53 (TP53) and β-catenin are the most frequently mutated tumor suppressor gene and oncogene in HCC, respectively.[11] The Janus Kinase (JAK)/STAT pathway and Wnt/β-catenin pathway may play roles in HCC acting as 2 major oncogenic drivers,[11] and Wnt/β-catenin pathway may cooperate with oxidative stress metabolism in HCC and ras/mitogen-activated protein kinase (MAPK) pathways.[12] Furthermore, microRNA-7 (miR-7), a tumor suppressor, can reverse the metastasis and inhibit the tumorigenesis of HCC by phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR)-signaling pathway.[13] microRNA-26a (MiR-26a) can suppress tumor growth and metastasis of HCC via targeting the interleukin-6-Stat3 pathway.[14] However, the molecular mechanisms leading to the development and progression of HCC have not been fully demonstrated.

Using the microarray data of GSE17548, a previous study indicated that senescence bypass played a central role in HCC engendering systematic changes,[15] and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC), cell cyclin D1 (CCND1), and amino acid metabolism pathways were important genes and pathways related to HCC, respectively.[2] In the present study, using the microarray data of GSE17548, we analyzed the differentially expressed genes (DEGs) in HCC tissues and compared them with those in cirrhosis tissues. Thereafter, pathway enrichment analyses for DEGs were performed and miRNA-target genes were predicted. Furthermore, a protein-protein interaction (PPI) network was constructed. We aimed to identify some pivotal genes and pathways for HCC transformed from cirrhosis, and explore potential targets for the treatment of the disease.

2. Methods

2.1. Microarray data

The microarray data of GSE17548 were downloaded from the Gene Expression Omnibus (GEO) database, which was based on the platform of GPL570 ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array, Affymetrix Inc, Ankara, Turkey) and deposited by Ozturk et al.[15] A total of 37 samples, including 20 cirrhosis samples and 17 HCC samples, were used for the analysis. Samples were derived from patients aged between 41 and 69 years, comprising more males than females. The etiology of these samples was hepatitis B virus (HBV), hepatitis C virus (HCV), or HBV+HDV (hepatitis D virus). Tissues in HCC samples were resected from inside the tumor, whereas those for cirrhosis samples were resected from adjacent tissue surrounding the tumor.

2.2. DEG analysis

The raw expression data were preprocessed by robust multiarray average (RMA)[16] algorithm using the Affy package in Bioconductor. After the preprocessing of the raw expression data, the DEGs in HCC tissues were compared with those in cirrhosis tissues and analyzed using the unpaired t test provided by limma package[17] in R/Bioconductor. |logFC| ≥1 and P value <.05 were considered as threshold values.

2.3. Pathway enrichment analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database used to map associated genes to their respective pathways.[18] ClueGO,[19] a Cytoscape plug-in, can decipher functionally grouped Gene Ontology (GO) and pathway annotation networks with a hypergeometric test, calculate the kappa coefficient[20] of pathways, and study functional correlations among pathways. CluePedia,[21] another Cytoscape plug-in, is a tool used to search new markers potentially related with pathways. Furthermore, ClueGO combined with CluePedia can derive better results. In the present study, KEGG pathway enrichment analysis was performed using ClueGO and CluePedia tool kits to study the functions of DEGs. A P value of <.05 and kappa coefficient of 0.4 were considered as threshold values. Furthermore, the key KEGG signaling pathway was analyzed using the R package pathview.[22]

2.4. Predictions of miRNA-target genes

The multiMiR R package[23] integrates miRNAs-target interactions with their disease and drug associations. The results of 14 databases were synthesized and merged into 3 categories including 3 verified miRNAs-target databases (miRecords, miRTarBase, and TarBase), 8 predicted miRNAs-target databases (DIANA-microT, ElMMo, MicroCosm, miRanda, miRDB, PicTar, PITA, and TargetScan), and 3 disease-/drug-related miRNA databases (miR2Disease, PharmacomiR, and PhenomiR) in this multiMiR R package.

The regulatory factor miRNA of DEGs was extracted from 3 verified miRNAs-target databases with multiMiR R package. We selected at least 2 verified relationship pairs to construct miRNA-DEGs regulatory networks. Furthermore, the gene ontology-biological process (GO-BP) was performed for target genes of key miRNA using ClueGO and CluePedia tool kits. A P value of <.05 was considered as the threshold value.

2.5. Construction of PPI network

The Search Tool for the Retrieval of Interacting Genes (STRING) database can provide predicted and experimental information of protein interactions.[24] The DEGs were mapped into PPIs and a combined score of >0.7 was identified as the cutoff standard. The PPI network was constructed using Cytoscape software.[25]
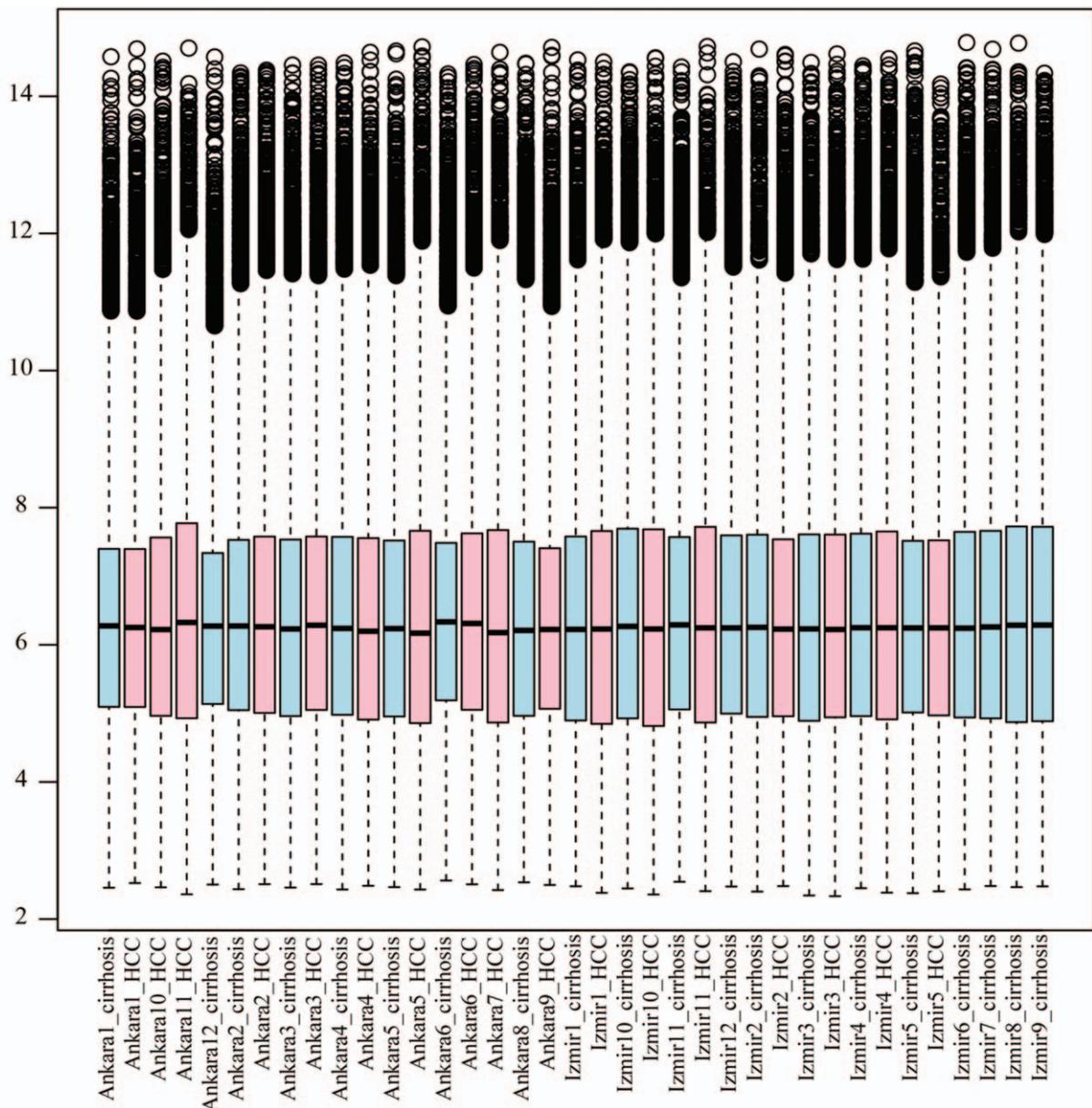
Figure 1. Boxplots of sample data. The lateral axis represents the names of samples, and the longitudinal axis represents the genes. Red represents hepatocellular carcinoma samples and blue represents cirrhosis samples.

3. Results

3.1. Data preprocessing

After background correction and normalization, a total of 20,108 genes were obtained in 37 HCC and cirrhosis tissue samples (Fig. 1).

3.2. DEGs and pathway enrichment analyses

As shown in Figure 2, a total of 224 DEGs including 77 downregulated and 147 upregulated genes (supplementary Table 1, http://links.lww.com/MD/B743) were identified in HCC tissues compared to in cirrhosis tissues, using |logFC| ≥1 and P value <.05 as threshold values. The function annotation of DEGs is shown in Figure 3. The significantly enriched pathways, by DEGs, included the cell cycle pathway, p53 signaling pathway,

caffeine metabolism pathway, primary bile acid biosynthesis pathway, bile secretion pathway, and retinol metabolism pathway. Furthermore, cyclin-dependent kinase 1 (*CDK1*) was enriched in the cell cycle pathway and p53 signaling pathway, and ribonucleotide reductase M2 (*RRM2*) was enriched in the p53 signaling pathway.

3.3. Regulatory networks of miRNA-DEGs

Most of the DEGs were regulated by hsa-miRNA-193b-3p, hsa-miRNA-192–5p, hsa-miRNA-26b-5p, and hsa-miRNA-335–5p (Fig. 4; supplementary Table 2, http://links.lww.com/MD/B744). The hsa-miRNA-26b-5p and hsa-miRNA-335–5p targeted DEGs were not significantly enriched in any GO-BP terms. The hsa-miRNA-192–5p targeted DEGs played a role in cytokinesis and sister chromatid segregation. The hsa-
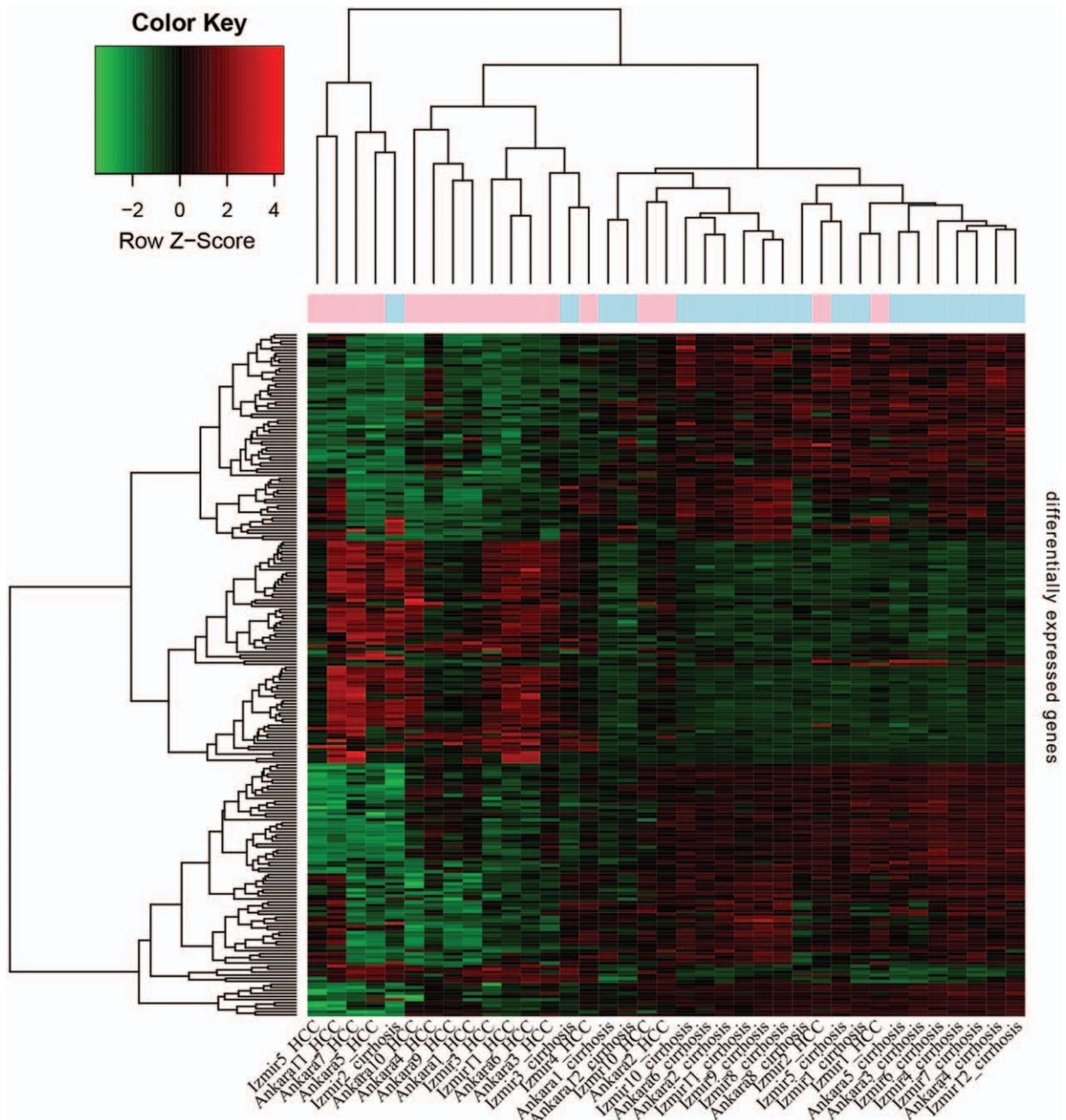
Figure 2. Heat maps of the differentially expressed genes in hepatocellular carcinoma tissues compared to in cirrhosis tissues. The lateral axis represents the names of samples, and the longitudinal axis represents the genes. Red represents hepatocellular carcinoma samples, whereas blue represents cirrhosis samples.

miRNA-193b-3p targeted DEGs also influenced microtube cytoskeleton organization involved in mitosis and sister chromatid segregation.

3.4. PPI network analysis

As shown in Figure 5, a total of 796 edges and 94 nodes were obtained based on STRING database, and a group of upregulated DEGs was gathered into a cluster. Furthermore, CDK1, PDZ-binding kinase (PBK), RRM2, and abnormal spindle homolog, microcephaly associated Drosophila (ASPM) were found to be the hub proteins with higher degrees in the PPI network.

4. Discussion

In the present study, a total of 224 DEGs, including 77 downregulated and 147 upregulated genes, were identified in HCC tissues compared to in cirrhosis tissues. Our results showed that CDK1, PBK, RRM2, and ASPM were the hub proteins with higher degrees in the PPI network. Furthermore, the cell cycle and p53 signaling pathways were significantly enriched by DEGs. In addition, *CDK1* was enriched in the cell cycle and p53 signaling pathways, and *RRM2* was enriched in the p53 signaling pathway.

A previous study showed that *CDK1* plays a role in the development of HCC by regulating the subcellular localization of
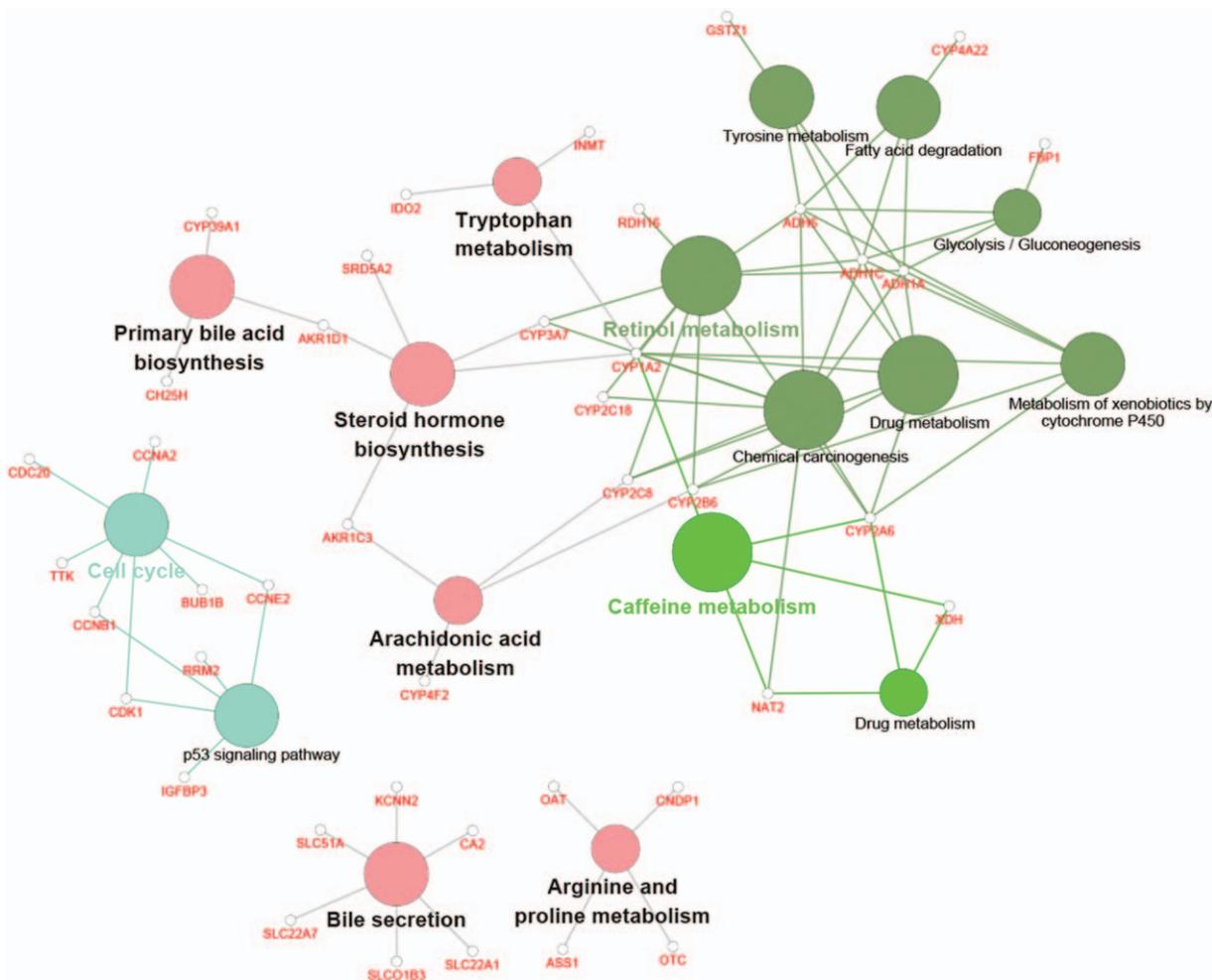
Figure 3. Function annotation of differentially expressed genes. Each node is a Kyoto Encyclopedia of Genes and Genomes pathway item. The node size reflects pathway significance (fdr): the smaller the fdr value, the larger the node size is. Edge between nodes reflects shared or common genes: the wider the edge, the larger the overlap is. Different node colors represent different functional groups. The most significant pathway of each group has a highlighted label.

apoptin.[26] Zhang et al[27] suggested that miR-582–5p regulated the development of HCC by inhibiting the expression of *CDK1*. A separate study also showed that *PBK* can promote tumor cell proliferation via regulation of the DNA damage response and p38 mitogen-activated protein kinase activity.[28] It is also known that high cell proliferation is closely associated with tumor recurrence in HCC.[29] SCY1-like 1-binding protein 1 (SCYL1-BP1) affects cell cycle arrest in HCC cells through RRM2 and Cyclin F.[30] Small-interfering RNA-mediated knockdown of *RRM2* can depress HCC cell proliferation.[31] Furthermore, Lee et al[32] indicated that high expression of *RRM2* could be a useful marker to predict early recurrence of HCC following curative hepatectomy. *ASPM* plays important roles in cell proliferation and neurogenesis, and enhanced invasive/metastatic potential in HCC can be predicted by *ASPM* overexpression.[33] Wurmbach et al[34] indicated that *ASPM* could be as a potential marker for early HCC. Therefore, our results are in line with previous findings and suggest that *CDK1*, *PBK*, *RRM2*, and *ASPM* may be key genes associated with the development of HCC.

In this study, cell cycle and p53 signaling pathways were significantly enriched by DEGs. The p110γ, one subclass of PI3K, can promote cell proliferation in HCC by controlling the cell cycle checkpoint.[35] Furthermore, miR-138 is a frequently downregulated miRNA in HCC, and can cause cell cycle arrest in HCC by targeting cyclin D3.[36] Besides, oleanolic acid can play inhibitory effects on HCC via cell cycle arrest and apoptosis mediated by extracellular signal–regulated kinase-p53 (ERK-p53).[37] Thus, the cell cycle pathway plays important mediatory roles in the development of HCC. The p53 signaling pathway is a potent barrier for tumor progression, and p53 plays important roles in hepatocarcinogenesis.[38,39] Moreover, p53 is a significant correlative regulatory factor of the α-fetoprotein (AFP) expression in HCC,[40] and Tu et al[41] found that the activation of F-box and WD repeat domain-containing 7 (Fbxw7) via adenoviral delivery of p53 caused increased proteasomal degradation of cyclin E and c-Myc, and thus recombinant human adenovirus-p53 injection could be a possible therapeutic agent for HCC. Thus, the p53 signaling pathway also plays significant mediatory roles for the progression of HCC. Therefore, our results are in line with those from previous studies and suggest that cell cycle and p53 signaling pathways play vital mediatory roles in the development of HCC.

Figure 4. Regulatory networks of miRNA-differentially expressed genes. Yellow border diamond node represents miRNA, whereas blue green border round node represents genes. Red nodes represent upregulation and green nodes represent downregulation. The lighter the nodes, the larger the degrees of differences and |logFC| are. The higher the degree, the bigger the size is.

As stated above, *CDK1* and *RRM2* may be key genes associated with the development of HCC, and cell cycle and p53 signaling pathways play vital roles in the development of HCC. Furthermore, in the present study, *CDK1* was enriched in cell cycle and p53 signaling pathways, whereas *RRM2* was enriched in the p53 signaling pathway. This suggests that *CDK1* affects HCC via its roles in the cell cycle and p53 signaling pathways, and *RRM2* affects HCC via its role in the p53 signaling pathway.

There were few limitations in the present study. Previous studies showed that the risk factors of HCC included hepatitis B and C infections, cirrhosis, and alcohol intake,[42] and cirrhosis was the largest risk factor, accounting for 80% to 90% of the total number of HCC cases.[43] In total, 1% to 1.5% of cirrhosis cases are transformed to HCC every year,[44] indicating the potential to identify molecular mechanisms associated with HCC via comparisons between HCC and cirrhosis. In this study, we performed bioinformatics analysis for microarray data, including 20 cirrhosis samples and 17 HCC samples. Several key genes and pathways were found to be important for HCC transformed from cirrhosis. In this study, we did not perform the analysis of the normal expression data, which, in addition to HCC and cirrhosis data, could provide more convincing findings. As such, we will carry out a comprehensive analysis including normal expression data, cirrhosis data, and HCC data in the future. Furthermore, we did not analyze other publicly available HCC data from GEO in parallel with adjacent normal tissues. A step-wise progressive analysis including other publicly available HCC data from normal to cirrhosis to HCC is needed in future studies.

5. Conclusions

In conclusion, *CDK1*, *PBK*, *RRM2*, and *ASPM* may be key genes for HCC transformed from cirrhosis. Furthermore, cell cycle and p53 signaling pathways play crucial roles in the development of HCC from cirrhosis. *CDK1* may play important roles in HCC transformed from cirrhosis via cell cycle and p53 signaling pathways, and *RRM2* may play a role in HCC transformed from cirrhosis via the p53 signaling pathway. However, owing to lack of experimental verification, normal expression data, and
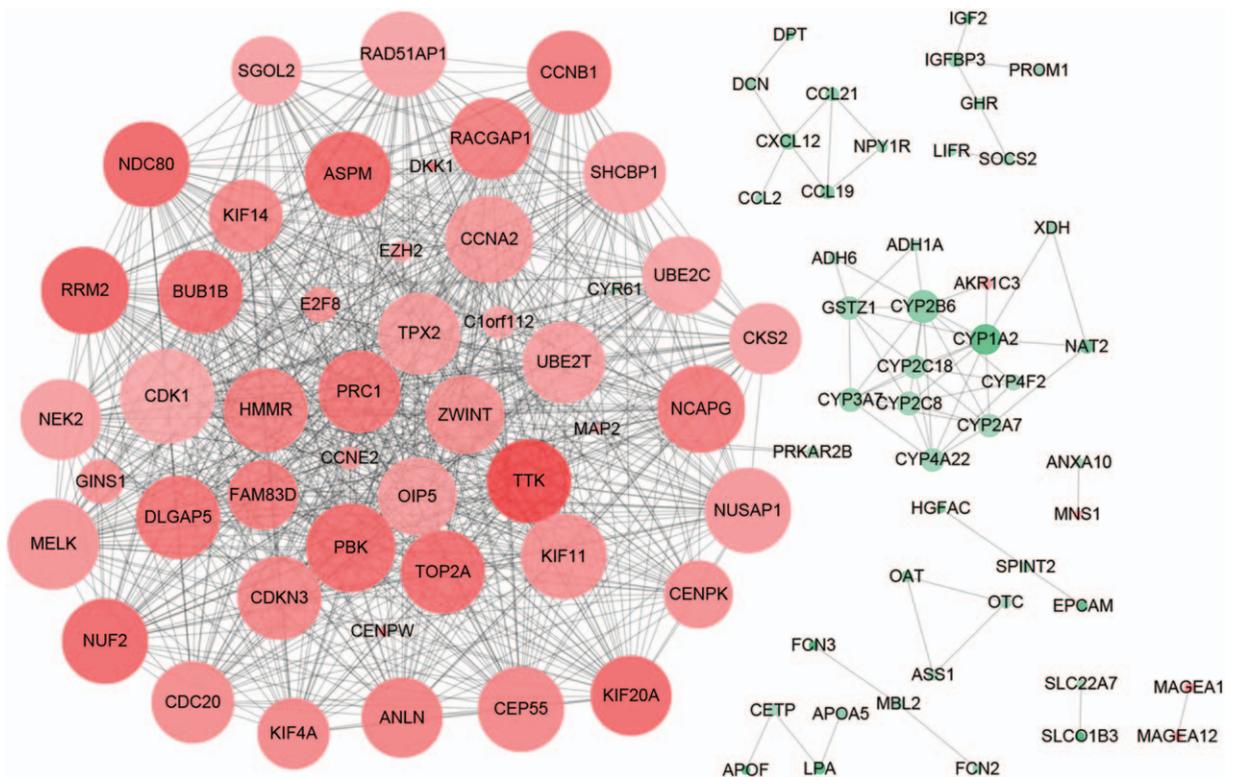
Figure 5. Protein-protein interaction network of differentially expressed genes. Red nodes represent upregulation, whereas green nodes represent downregulation. The lighter the nodes, the larger the degree of difference is. The higher the degree, the bigger the size is.

adequate sample size, further studies on the molecular mechanisms of HCC are needed.

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