From cirrhosis to hepatocellular carcinoma in HCV-infected patients: genes involved in tumor progression

Y. WANG, J. LI*, J. CHEN, L. LIU, Z. PENG, J. DING, K. DING

Department of Interventional Radiology, Fengxian Center Hospital, Shanghai (China)
*Emergency Department of Fengxian Center Hospital, Shanghai (China)

Abstract. – BACKGROUND, Hepatocellular carcinoma (HCC) is an aggressive tumor with poor prognosis. Understanding molecular changes in hepatocellular carcinoma should improve identification of risk factors in molecular subtypes and provide potential targets for early detection and therapy.

AIM, The present study aimed to analyze the molecular mechanism of the transition from hepatitis C virus (HCV) induced cirrhosis to HCV induced HCC using microarray analysis combined with bioinformatics techniques.

METHODS, To accomplish this, we performed the differential coexpression analysis of hepatic gene expression in samples of HCV-cirrhotic patients with and without HCC. Total 465 genes were identified and some of them were used to construct a regulatory network.

RESULTS, Our analysis indicated that several differentially co-express genes might play crucial roles in HCC development, including NA3C2, AHR, MYC, FOXO1 and FOSB. Further analysis predicted these genes might be involved in HCC through pathways of "ribosome", "steroid biosynthesis", "spliceosome" and so on. Moreover, these genes may serve as potential therapeutic targets for the treatment of HCC.

CONCLUSIONS, In conclusion, our findings confirm the presence of multiple molecular alterations during HCV-infected HCC hepatocarcinogenesis and indicate the possibility for identifying prognostic factors associated with HCC progression.

Key Words:
    Cirrhosis, Hepatocellular Carcinoma, Differentially co-express genes.

Introduction

Hepatocellular carcinoma (HCC) is a primary malignancy of the hepatocyte, generally leading to death within 6-20 months. More than 600,000 cases of liver cancer are diagnosed worldwide each year, and the number of new cases continues to grow[1]. The incidence of HCC worldwide varies according to the prevalence of hepatitis B and C infections. Areas such as Asia and sub-Saharan Africa with high rates of infectious hepatitis have prevalences as high as 120 cases per 100,000[2, 3]. Hepatocellular carcinoma frequently arises in the setting of cirrhosis, appearing 20-30 years following the initial insult to the liver[3,4]. Once cirrhosis has developed, retrospective studies have suggested that patients will develop either hepatic decompensation or HCC at a rate of 2% to 7% per year[5-7].

Substantial progress has been made over the years to identify major risk factors and to understand the pathogenesis of HCC. However, little is known about molecular mechanisms that lead to hepatocarcinogenesis. Changes occurring in liver tissues due to viral infection, exposure to hepatotoxic agents or other risk factors cause significant changes in the cellular signaling pathways and alter gene expression resulting in tumor formation[8]. Pathways such as Wnt/ β-Catenin pathway[9,10], p53 pathway[11,12], pRb pathway[13], mitogen-activated-protein-kinase (MAPK) pathway[14], Janus associated kinase/signal transducer and activator of transcription (JAK/STAT) pathway[15], epidermal growth factor receptor (EGFR) and transforming growth factor β (TGF-β) pathways were identified associated with liver cancer development. These pathways are being studied extensively to identify potential biomarkers and molecular targets.

The surveillance of patients at high risk of developing HCC is an important strategy that can potentially decrease the cancer-related mortality rate[16]. Therefore, the search for candidate biomarkers for HCC becomes one of hot topics in liver diseases for clinical researchers. The most commonly used serum marker of HCC is α-fetoprotein (AFP). However, its sensitivity is reported of 39% to 65% and specificity is reported of 65% to 94%, besides,

this serum marker has multiple limitations when applied to patients with hepatitis C virus (HCV)[17-19]. Furthermore, some researchers argue that it has limited utility as a screening test because it seems to have reduced sensitivity for smaller tumors[20]. Many researchers have identified and described genes that are uniquely up- or down-regulated in HCC tissues in recent years. Lee et al [21] suggested that cystatin B (CSTB) or the combination of CSTB and α-fetoprotein may be a useful marker for diagnosing patients with HCC with a high sensitivity. Noah et al[22] showed that serum cystatin C levels differ between patients with HCC compared with those with HCV cirrhosis and suggested cystatin C is one of the markers. Besides, Mas et al[16] observed differentially expressed angiogenesis genes between HCV patients with and without HCC and suggested soluble angiogenic factors might be useful for monitoring high-risk HCV patients, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), AGPTL2, ANG, EGFL6, EGFR, and so on.

The purpose of this study is to analyze the molecular mechanism of the transition from HCV induced cirrhosis to HCV induced HCC using microarray analysis combined with bioinformatics techniques. We sought to identify the differentially co-express genes in this progression and to find molecular markers for early detection of hepatocellular carcinoma of patients with cirrhosis.

Data and Methods

Affymetrix Microarray Data

The transcription profile of GSE17967 was obtained from a public functional genomics data repository Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) which are based on the Affymetrix GPL571 platform data (Affymetrix Human Genome U133A 2.0 Array). Only 63 chips are available for analysis, including 16 cirrhotic tissues from patients with HCV plus hepatocellular carcinoma and 47 cirrhotic tissues from HCV-positive patients who did not have concomitant hepatocellular carcinoma. There were no significant differences between HCV-positive patients with and without hepatocellular carcinoma on patient demographic characteristics, including patient age, gender, race, albumin, and alanine aminotransferase.

Pathway Data

KEGG (Kyoto Encyclopedia of Genes and Genomes) is one of the most popular pathway databases; it groups genes into pathways of interacting genes and substrates, and contains specific links between genes and substrates that interact directly[23,24]. The PATHWAY database records networks of molecular interactions in the cells, and variants of them specific to particular organisms (http://www.genome.jp/kegg/).

Regulation Data

UCSC (http://genome.ucsc.edu) is an interactive website offering access to genome sequence data from a variety of vertebrate and invertebrate species and major model organisms, integrated with a large collection of aligned annotations. We downloaded the human transcription and chromosome region from UCSC.

Differential Co-Expression Analysis (DCEA)

DCEA is increasingly used for investigating the global transcriptional mechanisms underlying phenotypic changes. DCEA is developed to examine the change in gene expression correlation between two conditions, which is accordingly designed as a complementary technique to traditional differential expression analysis[25-27].

For GSE17967, the derived concentration guidelines level (DCGL) package in R[28] was used to perform differential co-expression analysis between cirrhotic patients with and without HCC. We calculated the p-value and adjusted the raw p-value into q-value. The genes only with q-value less than 0.25 were selected as differentially co-express genes (DCGs).

Regulation Network Construction

Using the regulation data that have been collected from UCSC, we matched the relationships between differentially co-express genes and its target genes. Base on the above regulation datasets, we built the regulation network by Cytoscape[29].

Results

Microarray Data Analysis

Publicly available microarray data set GSE17967 was obtained from GEO. Total 465 DCGs with the q-value < 0.25 were selected using the DCGL method in R.

Regulatory Network Construction in HCC

Total 465 DCGs were selected using the DCGL method (Figure 1). We matched the relationships

between differentially co-expressed genes and their target genes using the regulation data that have been collected from UCSC. We got 24 pairs of relationships between transcription factors (TFs) and their target genes in the progression from cirrhosis to HCC. We suggested that these 24 pairs of relationships play an important role in the transition from cirrhosis to HCC in the expression level. In this network, the TF NR3C2, AHR, MYC, FOXO1, FOSB with high degree form a local network which suggesting these TFs may play an important role in the development of this disease. Total 23 genes which acted as target genes and regulated by the above TFs were identified in our network. The target genes include CD46, ATP5H, HSPE1, PER1, XBP1, and TGDS and so on.

Pathway Enrichment Analysis of the Differentially Co-Express Genes

Pathway can provide an alternative way to relax the significance threshold applied to single genes and may lead to a better biological interpretation[30]. In order to facilitate the functional annotation and analysis of large lists of genes in our result and to identify the relevant pathways changed in progression from cirrhosis to HCC, we performed pathway enrichment analysis to the differentially co-express genes used a statistical approach (Table I). The pathway enrichment analysis yields many significant pathways contained ribosome, steroid biosynthesis, spliceosome, NOD-like receptor signaling pathway and so on.
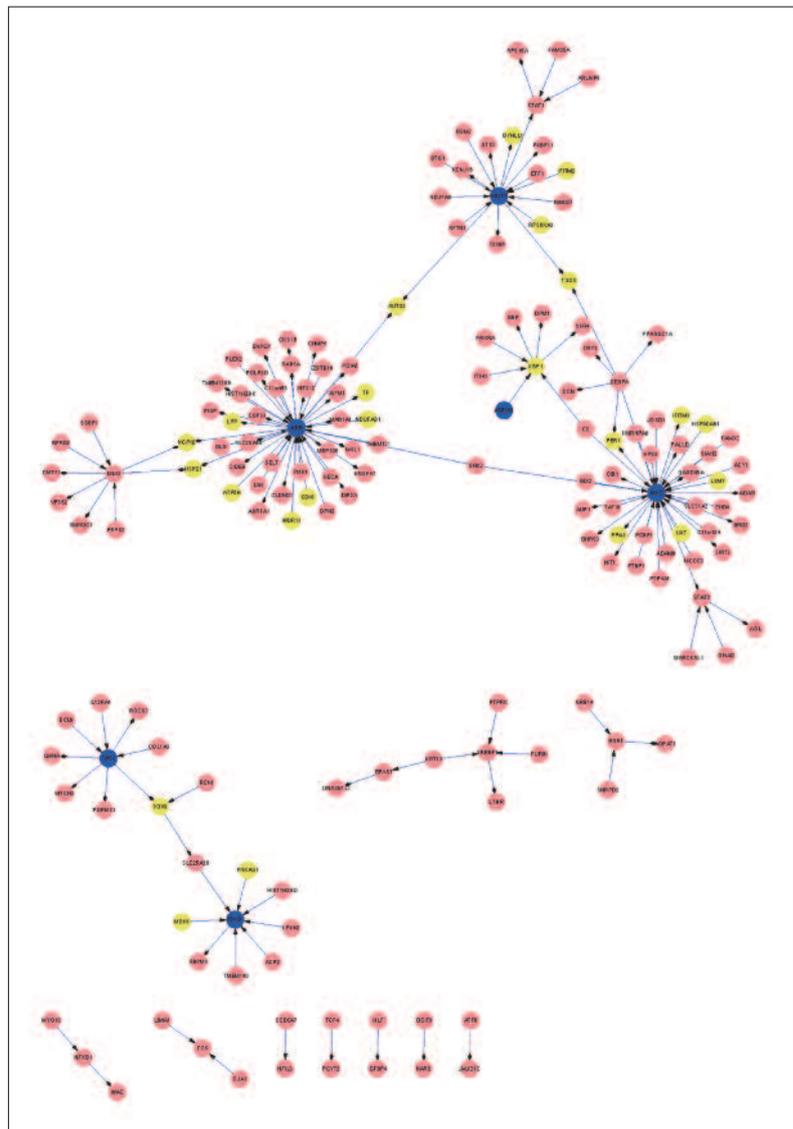

Figure 1. Regulatory network construction in HCC. The blue node indicates that the gene is not only a DCG but also a TF. The yellow node indicates that the gene is not only DCG but also a target gene. The red node indicates that the gene has a regulatory relationship with the TF, but the corresponding TF is not a DCG.

Discussion

The growing incidence of HCC has generated intense research to understand the pathogenesis, cellular, and molecular mechanisms of the disease with the hope of developing new treatment strategies. Due to the low efficiency of current therapy of patients with hepatitis C induced cirrhosis that are diagnosed with advanced-stage hepatocellular carcinoma, the search for improved markers as potential therapeutic targets and for the early detection has become a priority. However, detection of liver cirrhosis and hepatocellular cancer is often difficult in chronic hepatitis C infected patients[17]. The present study, therefore, aimed to analyse the molecular mechanism of the transition from HCV-infected cirrhosis to HCV- infected HCC using microarray analysis combined with bioinformatics techniques. We sought to identify the differentially expressed genes in this progression and to find molecular markers for early detection of hepatocellular carcinoma of patients with cirrhosis.

Studying the gene profiles in samples of HCV-cirrhotic patients with and without HCC using the DCGL package in R, we were able to identify a series of differentially co-express genes. Specifically, when samples from HCV-cirrhotic patients were compared to HCV-HCC samples, 465 genes were significantly differentially co-express between groups (q-value < 0.25)

From the regulatory network constructed in our result, the TF NR3C2, AHR, MYC, FOXO1, FOSB with high degree form a local network which suggesting these TFs play an important role in the development of this disease. We would discuss the relationship between HCC and identified genes as follows based on previous reports.

NR3C2 (nuclear receptor subfamily 3, group C, member 2) encodes the mineralocorticoid receptor, which mediates aldosterone actions on salt and water balance within restricted target cells. A role for miR-124 in the regulation of invasion and metastasis in the molecular aetiology of HCC has been previously published[31]. Previous study have demonstrated that miR-124 could participate in the regulation of renin-angiotensin system (RAS) which involved in many types of cancer[32] by repressing the mineralocorticoid receptor gene NR3C2[33]. Therefore, the gene NR3C2 might play an important role in the development of HCC.

AHR (aryl hydrocarbon receptor) encodes a cytosolic DNA binding protein which mediates the immunotoxicity of xenobiotics such as 2,3,7,8-tetrachlorodibenzo-p-dioxin-(TCDD), and targets inactivation produces abnormal immune system development[34]. The AHR is a ligand-activated transcription factor that plays an important role in cell cycle regulation and apoptosis[35] and functions as a modulator of cellular signaling pathways[36]. Many AHR ligands activate signaling cascades initiated and propagated. Transduction of such signals to the nucleus induces the expression of multiple immediate-early response genes, including myeloblastosis (MYB), myelocytomatosis (MYC), and members of the FOS and JUN families[37]. This early response explains the ability of TCDD and other ligands of the AHR act as powerful tumor promoters and carcinogens. Base on the studies above, we can conclude that AHR might also play an important role in the hepatocarcinogenesis.

The Myc family proteins are thought to play a crucial role in cellular proliferation, differentiation, transformation, and apoptosis. Mutations, overexpression, rearrangement and translocation of this gene have been associated with a variety of hematopoietic tumors, leukemias and lymphomas. C-Myc is a known inducer of wild type p53, decreased c-myc expression may lead to uncontrolled cell growth because of the lack of p53 expression that normally induces apoptosis[38]. A recent study

Table I. The top 10 enriched KEGG pathways.

KEGGID	p-value	Count	Size	Term
3010	2.21E-06	13	88	Ribosome
100	0.001496	4	17	Steroid biosynthesis
3040	0.001795	11	128	Spliceosome
4621	0.002702	7	62	NOD-like receptor signaling pathway
4610	0.018662	6	69	Complement and coagulation cascades
5215	0.019005	7	89	Prostate cancer
980	0.021211	6	71	Metabolism of xenobiotics by cytochrome P450
982	0.023986	6	73	Drug metabolism - cytochrome P450
140	0.027895	5	56	Steroid hormone biosynthesis
72	0.029307	2	9	Synthesis and degradation of ketone bodies

demonstrated that temporary inhibition of Myc selectively kills mouse lung cancer cells, suggesting Myc is a potential cancer drug target[39].

FOXO1 (Forkhead box O1), a member of the Forkhead box-containing O family of transcription factors, is a key regulator of diverse cellular functions, such as cell differentiation, proliferation, homeostasis, metabolism, and survival[40]. There has been a vast increase in evidence that the deregulation of Fox family transcription factors has a crucial role in the development and progression of cancer during the past years. The role of Fox proteins as direct and indirect targets for therapeutic intervention, as well as biomarkers for predicting and monitoring treatment responses is emerging these years[41]. In our regulatory network, FOXO1 acted as a hub node which regulates a set of genes. Our finding indicates that FOXO1 may be involved to hepatocarcinogenesis on the stage where cirrhosis progress into HCC. This indication is in line with previous study.

FBJ urine osteosarcoma viral oncogene homolog B (FOSB), a member of activator protein-1 (AP-1) family, encodes leucine zipper proteins that can dimerize with proteins of the JUN family. AP-1 lies at the nexus of many signaling pathways and transduces extracellular stimuli such as growth factors, cytokines, and environmental stress, modulating a variety of biological processes, including cell growth, death, differentiation, and oncogenic transformation[42,43]. In our regulatory network, FOSB regulates a set of genes, such as interleukin 13 receptor, α1 (IL13RA1), B-cell limpoma 6 (BCL6), collagen α-2(1) (COL1A2), etc., which suggesting FOSB play an important role in the development of HCC from cirrhosis. This finding is in concordance with a previous publication of Mas et al[44].

In conclusion, we employed microarray analysis combined with bioinformatics techniques to compare differential gene expression between HCV-cirrhosis and HCV-HCC to study the molecular mechanism of this disease. The differentially co-express genes included genes related to regulation of cell proliferation, differentiation, and transformation as FOXO1, FOSB and MYC, indicating a possible association between HCV- infected HCC and the cell proliferation. Besides, we also identified some new potential therapeutic targets genes, such as NA3C2 and AHR. Further, we performed pathway enrichment analysis to these differentially co-express genes and found that some of these genes might play roles in the progression from cirrhosis to HCC through pathways of "ribosome", "steroid biosyn-

thesis", "spliceosome" and so on. Further research surrounding the role of genes in our result in the progression from cirrhosis to hepatocellular carcinoma is certainly needed, considering the lack of effective therapies currently available.

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