ORIGINAL ARTICLE

Bioinformatics microarray analysis and identification of gene expression profiles associated with cirrhotic liver

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Abstract   Cirrhosis is the endpoint of liver fibrosis that is accompanied by limited regeneration capacity and complications and is the ultimate cause of death in many patients. Despite this, few studies have thoroughly looked at the gene expression profiles in the cirrhotic liver. Hence, this study aims to identify the genes that were differentially expressed in the cirrhotic liver and to explore the putative related signaling pathway and interaction networks. The gene expression profiles of cirrhotic livers and noncirrhotic livers were examined and compared using microarray gene analysis. Proteins encoded by the differentially expressed genes were analyzed for functional clustering and signaling pathway involvement using MetaCore bioinformatics analyses. The Gene Ontology analysis as well as the Kyoto encyclopedia of Genes and Genomes pathway analysis were also performed. A total of 213 significant genes were differentially expressed at more than a two-fold change in cirrhotic livers as compared to noncirrhotic livers. Of these, 105 upregulated genes and 63 downregulated genes were validated through MetaCore bioinformatics analyses. The signaling pathways and major functions of proteins encoded by these differentially expressed genes were further analyzed; results showed that the cirrhotic liver has a unique gene expression pattern related to inflammatory reaction, immune response, and cell growth, and is potentially cancer related. Our findings suggest that the microarray analysis may provide clues to the molecular mechanisms of liver cirrhosis for future experimental studies. However, further exploration of areas regarding therapeutic strategy might be possible to support metabolic activity, decrease inflammation, or enhance regeneration for liver cirrhosis.

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Introduction

The liver is a quiescent organ in the adult body that has the unique capacity to regulate its growth and regenerate after injury and partial hepatectomy. This property is particularly remarkable in clinical circumstances such as toxic injury, viral hepatitis, and hepatectomy, situations in which quiescent hepatocytes proliferate and replicate to restore the mass and functional capacity of the liver [1,2]. However, the regenerative ability of a cirrhotic liver is relatively limited because of diffuse fibrosis of hepatic parenchyma, which also prohibits liver resection in patients with liver cirrhosis [3,4]. Currently, liver cirrhosis is always associated with hepatocellular carcinoma (HCC), and both are leading causes of death worldwide [5–8]. Although liver resection remains the usual course of treatment for patients with HCC, the reduced regeneration capacity would limit the benefit of liver resection for patients with hepatic malignancy [9–11].

Liver cirrhosis is an advanced stage of liver fibrosis that results when the normal wound-healing response leads to an abnormal continuation of connective tissue production and deposition [12]. The wound-healing response produces a formation of scar tissue that is composed of a complex assembly of different extracellular matrix (ECM) molecules [13]. Additionally, a growing number of changes in genetic expressions that likely affected fibrosis progression had been described [14–16]. However, the majority of candidate differentially expressed genes need to be confirmed further. Therefore, this study collected liver tissues from cirrhotic and noncirrhotic livers and compared gene expression using microarray technology to identify gene expression differences in cirrhotic livers compared to noncirrhotic livers.

Methods

Patients

All study procedures and protocols were approved by the Institutional Review Boards of Chang Gung Memorial Hospital, Taoyuan, Taiwan. Patients who underwent liver resection at the Department of General Surgery at the Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan, were screened for inclusion in this study between December 2011 and December 2013. Written informed consent was obtained from all patients prior to the operation, and 40 patients were enrolled in this study. Liver tissue was obtained through wedge liver biopsies during operation. In case of hepatic malignancy, tissue samples were taken from the part of the liver that did not contain the tumor. All liver tissues were initially subjected for histological examination, and the Ishak fibrosis score was used to assess the cirrhosis status of liver parenchyma [17]. Patients who had Ishak fibrosis score ≥ 5 (marked portal—portal and/or portal central bridging with occasional nodules) were defined as liver cirrhosis cases.

Based on the classification, patients were categorized into two groups: cirrhotic liver ($n = 24$) and noncirrhotic liver ($n = 16$). The clinical characteristics of patients are listed in Table 1. The cirrhotic group consisted of 10 patients who had undergone liver resection because of HCC with ($n = 9$) or without ($n = 1$) virus hepatitis, and 14 patients who had liver transplantation. The indications of liver transplantation were virus hepatitis-related cirrhosis with ($n = 7$) or without HCC ($n = 5$), Wilson disease ($n = 1$), and unknown etiology of end-stage liver cirrhosis ($n = 1$). None of these patients had alcoholic-related liver cirrhosis. The noncirrhotic group consisted of four living-related liver donors and 12 patients who had liver resection because of liver tumor including HCC ($n = 3$), hemangioma ($n = 1$), and colorectal cancer hepatic metastasis ($n = 8$).

Tissue preservation and RNA extraction

Liver biopsies were placed immediately in RNAlater RNA Stabilization Reagent (Qiagen Sciences, Valencia, CA, USA) and frozen at $-20°C$. Total RNA was extracted from the liver biopsies and isolated using the RNeasy Mini Kit (Qiagen Sciences), according to the manufacturer's protocol. Contaminating genomic DNA was removed using gDNA Eliminator columns from the RNeasy Mini Kit (Qiagen Sciences). Then, first-strand cDNA was synthesized from 2 μg of total RNA by using the Super-Script first-strand synthesis system (Invitrogen, Carlsbad, CA, USA).

Microarray and data processing

The first-strand cDNA converted from mRNA was fragmented into cRNA using T7 RNA polymerase with biotinylated nucleotides (Promega, Madison, WI, USA). Then, 15 μg of fragmented cRNA was hybridized to each Affymetrix HuGene 2.0 Chip (Affymetrix, Santa Clara, CA, USA). Chips were hybridized, washed, and stained as per the Affymetrix standard protocol, and signal intensities corresponding to gene expression were generated through the Affymetrix GeneChip Operating Software (GCOS).

Table 1 Clinical demographic and characteristic of patients.

Characteristics	Cirrhosis ($n = 24$)	Noncirrhosis ($n = 16$)	p
Age (y)	57 (33–76)	54 (31–69)	0.198
Sex			0.502
Male	15 (63)	12 (75)	
Female	9 (37)	4 (25)	
Hepatitis status			<0.0001
Hepatitis B virus	16 (67)	1 (6)	
Hepatitis C virus	4 (17)	0	
Hepatitis B & C	1 (4)	0	
None	3 (12)	15 (94)	
Liver tumor			0.888
Primary HCC	17 (71)	3 (19)	
CRC metastasis	0	8 (50)	
Hemangioma	0	1 (6)	
No	7 (29)	4 (25)	

Data are presented as n (%) or median (range).
CRC = colorectal cancer; HCC = hepatocellular carcinoma.