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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.

Conflicts of interest: All authors declare no conflicts of interest.

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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 $\geq$ 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.

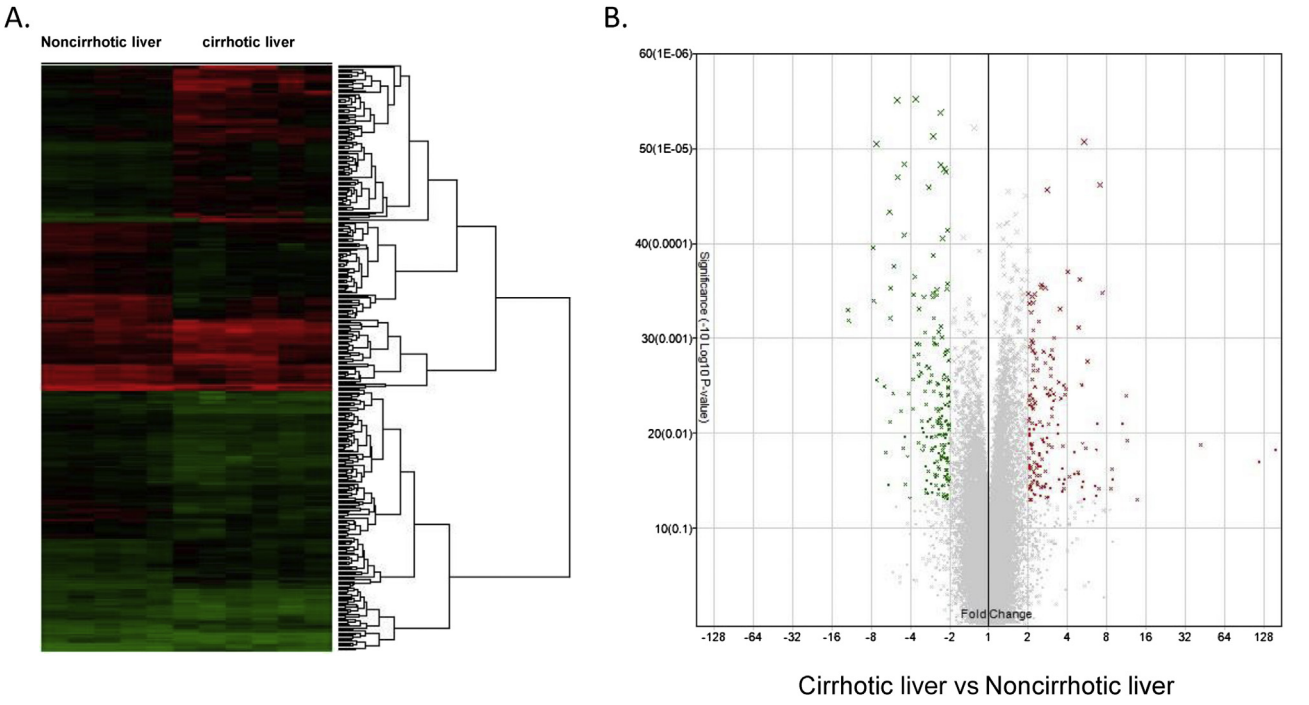

Figure 1. Microarray gene analysis. (A) Heat map of gene expression profiles. Red color indicates upregulation; green color represents downregulation. (B) Volcano plot of differentially expressed genes. Red color indicates upregulation; green color represents downregulation. Genes with a significant change of more than two-fold were selected.

Microarray gene profiles were obtained from each liver sample, and significant differences of expression gene were analyzed using group comparison. Genes that were statistically significant with a p value < 0.05 as well as expressed at two-fold or greater difference between the two groups were selected for further analysis. The biological function analysis of gene-encoded protein was done using MetaCore (Life Sciences Research, Thomson Reuters, UK) analysis or the Database for Annotation, Visualization, and Integrated Discovery (DAVID; david.ncifcrf.gov) [18]. Pathway analysis and additional analysis of gene function was performed using the Gene Ontology (GO) analysis and/or Kyoto encyclopedia of Genes and Genomes (KEGG) pathway analysis.

Results

Differential gene expression profiles

In order to clarify differential gene expression between cirrhotic and noncirrhotic livers, gene expression levels were analyzed in the two groups. Genes with a significant change of two-fold or more were screened, yielding 127 upregulated genes and 86 downregulated genes from the microarray analysis (Figure 1). Subsequently, these genes were further analyzed and categorized by MetaCore analysis. After excluding unknown genes, 105 upregulated genes (Table 2) and 63 downregulated genes were validated (Table 3). Based on their putative functions, genes were categorized into subgroups including transcription factors ($n = 5$), receptors ($n = 9$), ligands ($n = 8$), kinases ($n = 3$), proteases ($n = 10$), phosphatases ($n = 3$), generic enzymes ($n = 47$), proteins and binding proteins ($n = 64$), and others ($n = 19$).

Function and pathway of encoded proteins

To determine the function and pathway of the protein encoded by each gene, a MetaCore search for each gene were performed. Although numerous genes have multiple functions, this study focused on the major pathway and function of the protein encoded by the gene. For genes that play a major role in inflammation and immunologic reaction, 18 genes were upregulated, but only one gene was downregulated in the cirrhotic liver compared to the noncirrhotic liver. Table 4 shows the pathway analysis results in terms of inflammation and immunologic reaction. The upregulated inflammatory and immunologic genes included genes involved in numerous inflammation reactions such as vascular inflammation, regulation of cytokines, and activation of chemokine in the cirrhotic liver.

Additionally, there were 19 significantly upregulated genes involved in the cell cycle in tasks such as cell differentiation, cell division, growth regulation, wound healing, and apoptosis in the cirrhotic liver compared to the noncirrhotic liver, demonstrating that the cirrhotic liver was undergoing self-repair. However, the frequent cycle of parenchymal damage and repair has led to scar formation

Table 2 Upregulated gene expression of cirrhotic liver as compared with noncirrhotic liver.

Gene symbol	Gene name	Fold change	*p*
Transcription factors			
STAT1	Signal transducer & activator of transcription 1	2.6	0.001
ZNF215	Zinc finger protein 215	2.13	0.002
NCKAP1L	NCK-associated protein 1-like	2.13	0.007
ELF3	E74-like factor 3	2.08	0.036
Receptors			
OSMR	Oncostatin M receptor	2.97	0.002
LGALS3BP	Lectin, galactoside-binding, soluble, 3 binding protein	2.53	0.002
HLA-A	Major histocompatibility complex, class I, A	2.25	0.043
HLA-DRB3	Major histocompatibility complex, class II, DR beta 3	2.16	0.005
ITGA2	Integrin, alpha 2	2.1	0.049
Ligands			
CCL20	Chemokine (C—C motif) ligand 20	5.15	0.037
SPP1	Secreted phosphoprotein 1	4.5	0.026
CXCL6	Chemokine (C—X—C motif) ligand 6	3.37	0.008
GDF15	Growth differentiation factor 15	2.34	0.034
LAMA2	Laminin, alpha 2	2.32	0.027
A2M	Alpha-2-macroglobulin	2.24	<0.001
JAG1	Jagged 1	2.19	0.024
IL32	Interleukin 32	2.04	0.010
Kinase			
HKDC1	Hexokinase domain containing 1	4.86	<0.001
CHEK2	Checkpoint kinase 2	2.62	0.012
Protease			
MMP7	Matrix metallopeptidase 7	2.69	0.025
TMPRSS3	Transmembrane protease, serine 3	2.26	0.001
TMPRSS4	Transmembrane protease, serine 4	2.26	0.001
HTRA1	HtrA serine peptidase 1	2.18	0.002
BACE2	Beta-site APP-cleaving enzyme 2	2.04	0.021
Phosphatase			
SGPP1	Sphingosine-1-phosphate phosphatase 1	4.5	0.026
PLA2G2A	Phospholipase A2, group IIA	4.11	0.028
Generic enzymes			
GGTLC2	Gamma-glutamyltransferase light chain 2	6.49	0.035
ACSL4	Acyl-CoA synthetase long-chain family member 4	5.52	0.014
UPP2	Uridine phosphorylase 2	5.22	0.012
LIPH	Lipase, member H	4.95	<0.001
LIPI	Lipase, member I	4.95	<0.001
ENPP2	Ectonucleotide pyrophosphatase/phosphodiesterase 2	4.02	<0.001
NQO1	NAD(P)H dehydrogenase, quinone 1	3.64	0.040
CHST9	Carbohydrate (*N*-acetylgalactosamine 4-0) sulfotransferase 9	3.03	0.006
ENPP5	Ectonucleotide pyrophosphatase/phosphodiesterase 5	3.02	0.005
APOBEC3C	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C	2.78	<0.001
TYMS	Thymidylate synthetase	2.72	0.034
GPX2	Glutathione peroxidase 2	2.7	0.001
FAM111B	Family with sequence similarity 111, member B	2.66	0.036
CDS1	CDP-diacylglycerol synthase 1	2.62	0.012
TUSC3	Tumor suppressor candidate 3	2.43	0.017
NEDD4L	Neural precursor cell expressed, developmentally downregulated 4-like	2.4	<0.001
OAS2	2′-5′-Oligoadenylate synthetase 2	2.31	0.001
IFI30	Interferon, gamma-inducible protein 30	2.24	0.004
GLS	Glutaminase	2.23	0.020
GLS2	Glutaminase 2	2.23	0.020
UBASH3A	Ubiquitin associated & SH3 domain containing A	2.18	0.002
TGM2	Transglutaminase 2	2.18	0.015
GCNT4	Glucosaminyl (*N*-acetyl) transferase 4, core 2	2.18	0.001
TMEM55A	Transmembrane protein 55A	2.17	0.001
ACSM1	Acyl-CoA synthetase medium-chain family member 1	2.12	0.005

Table 2 (*continued*)

Gene symbol	Gene name	Fold change	*p*
MOXD1	Monooxygenase, DBH-like 1	2.1	0.034
MX1	Myxovirus (influenza virus) resistance 1	2.07	0.013
Protein, binding protein			
TMEM45B	Transmembrane protein 45B	7.07	<0.001
EPCAM	Epithelial cell adhesion molecule	6.72	0.007
POF1B	Oremature ovarian failure, 1B	5.66	0.001
FAT1	FAT tumor suppressor homolog 1	5.12	0.003
FABP4	Fatty acid binding protein 4, adipocyte	4.83	0.025
CXXC1	CXXC finger protein 1	4.5	0.026
GPC3	Glypican 3	4.49	0.015
DTNA	Dystrobrevin, alpha	3.81	0.003
LGALS3	Lectin, galactoside-binding, soluble, 3	3.72	0.003
IGLV1-40	Immunoglobulin lambda variable 1—40	3.43	0.030
TSPAN8	Tetraspanin 8	3.41	0.002
SLPI	Secretory leukocyte peptidase inhibitor	3.16	<0.001
GOLM1	Golgi membrane protein 1	3.1	0.001
DCDC2	Doublecortin domain containing 2	3.02	0.013
IFI6	Interferon, alpha-inducible protein 6	3.01	0.001
LRRC1	Leucine rich repeat containing 1	2.94	0.001
FAM169A	Family with sequence similarity 169, member A	2.92	0.003
TIMP1	TIMP metallopeptidase inhibitor 1	2.85	0.013
LUM	Lumican	2.84	0.046
SEL1L3	Sel-1 suppressor of lin-12-like 3	2.57	0.008
IGHV4-31	Immunoglobulin heavy variable 4-31	2.57	0.011
GPRIN3	GPRIN family member 3	2.5	<0.001
ANXA2P2	Annexin A2 pseudogene 2	2.47	0.022
HSPA4L	Heat shock 70 kDa protein 4-like	2.44	0.007
CD24	CD24 molecule	2.31	0.023
HLA-A	Major histocompatibility complex, class I, A	2.25	0.043
A2M	Alpha-2-macroglobulin	2.24	<0.001
CDHR2	Cadherin-related family member 2	2.19	0.026
MMVP1	Myxomatous mitral valve prolapse 1	2.19	<0.001
MVP	Major vault protein	2.19	<0.001
CLIP4	CAP-GLY domain containing linker protein family, member 4	2.18	0.002
TMEM87B	Transmembrane protein 87B	2.14	<0.001
TTC9	Tetratricopeptide repeat domain 9	2.13	0.014
LXN	Latexin	2.12	0.039
SPTBN1	Spectrin, beta, nonerythrocytic 1	2.08	0.036
PTGFRN	Prostaglandin F2 receptor negative regulator	2.06	0.001
RASSF3	Ras association (RalGDS/AF-6) domain family member 3	2.05	0.023
RASSF5	Ras association (RalGDS/AF-6) domain family member 5	2.05	0.023
ID4	Inhibitor of DNA binding 4, dominant negative helix—loop—helix protein	2.04	0.024
LOXL4	Lysyl oxidase-like 4	2.03	0.010
EZR	Ezrin	2.02	0.007
CDH6	Cadherin 6, type 2, K-cadherin	2.01	0.018
Others			
SORT1	Sortilin 1	5.33	<0.001
SLC6A11	Solute carrier family 6, member 11	3.86	0.003
SLC38A1	Solute carrier family 38, member 1	3.5	<0.001
GPR64	G protein-coupled receptor 64	2.77	0.011
SLC35C1	Solute carrier family 35, member C1	2.69	<0.001
SLC22A15	Solute carrier family 22, member 15	2.54	0.018
CFTR	Cystic fibrosis transmembrane conductance regulator	2.48	0.045
F2RL1	Coagulation factor II (thrombin) receptor-like 1	2.19	0.005
RASD1	RAS, dexamethasone-induced 1	2.07	0.038
SLC12A2	Solute carrier family 12 (sodium/potassium/chloride transporters), member 2	2.05	0.049

Table 3 Downregulated gene expression of cirrhotic liver as compared with noncirrhotic liver.

Gene symbol	Gene name	Fold change	*p*
Transcription factor			
PHOX2A	Paired-like homeobox 2a	3.83	0.002
Receptors			
ABCC9	ATP-binding cassette, sub-family C, member 9	2.44	0.003
IL1RAP	Interleukin 1 receptor accessory protein	2.29	0.005
PTPRD	Protein tyrosine phosphatase, receptor type, D	2.1	0.024
PTPRS	Protein tyrosine phosphatase, receptor type, S	2.1	0.024
Kinase			
MAP2K6	Mitogen-activated protein kinase kinase 6	2.65	0.003
Protease			
CNDP1	Carnosine dipeptidase 1	7.24	0.002
FOLH1B	Folate hydrolase 1B	3.29	0.007
MMP12	Matrix metallopeptidase 12	3.1	0.003
MME	Membrane metallo-endopeptidase	3.1	0.003
NAALAD2	*N*-Acetylated alpha-linked acidic dipeptidase 2	2.07	0.030
Phosphatase			
ENPP3	Ectonucleotide pyrophosphatase/phosphodiesterase 3	3.0	0.001
Generic enzymes			
SRD5A2	Steroid-5-alpha-reductase, alpha polypeptide 2	6.35	0.003
CYP7A1	Cytochrome P450, family 7, subfamily A, polypeptide 1	4.56	0.014
SULT1E1	Sulfotransferase family 1E, estrogen-preferring, member 1	4.43	<0.001
LGSN	Lengsin, lens protein with glutamine synthetase domain	4.2	0.017
ASPA	Aspartoacylase	3.76	0.001
CYP2C19	Cytochrome P450, family 2, subfamily C, polypeptide 19	3.47	0.001
BCHE	Butyrylcholinesterase	3.31	0.002
CYP1A1	Cytochrome P450, family 1, subfamily A, polypeptide 1	3.21	0.015
CYP1A2	Cytochrome P450, family 1, subfamily A, polypeptide 2	3.21	0.015
BBOX1	Butyrobetaine, 2-oxoglutarate dioxygenase 1	2.82	0.007
HSD17B14	Hydroxysteroid (17-beta) dehydrogenase 14	2.67	<0.001
DHRS2	Dehydrogenase/reductase (SDR family) member 2	2.5	0.001
OAT	Ornithine aminotransferase	2.5	0.019
CYP4A22	Cytochrome P450, family 4, subfamily A, polypeptide 22	2.39	0.023
EPHX2	Epoxide hydrolase 2, cytoplasmic	2.32	0.008
ACSM3	Acyl-CoA synthetase medium-chain family member 3	2.24	0.013
CYP4A11	Cytochrome P450, family 4, subfamily A, polypeptide 11	2.24	0.012
GPAM	Glycerol-3-phosphate acyltransferase, mitochondrial	2.23	0.043
ADCY10	Adenylate cyclase 10	2.2	0.028
STEAP1	Six transmembrane epithelial antigen of the prostate 1	2.12	0.045
Protein, binding protein			
C5orf27	Chromosome 5 open reading frame 27	11.82	<0.001
TRIM55	Tripartite motif containing 55	7.31	<0.001
RANBP3L	RAN binding protein 3-like	5.73	0.007
CTNNA3	Catenin (cadherin-associated protein), alpha 3	4.45	<0.001
NCAM2	Neural cell adhesion molecule 2	3.83	0.002
IDO2	Indoleamine 2,3-dioxygenase 2	3.46	0.012
FAM106CP	Family with sequence similarity 06, member C, pseudogene	3.1	<0.001
MT1G	Metallothionein 1G	3.0	0.031
PPP1R1A	Protein phosphatase 1, regulatory subunit 1A	3.0	0.010
LPA	Lipoprotein, Lp(a)	2.77	0.033
CCDC144A	Coiled-coil domain containing 144A	2.55	0.010
SLITRK3	SLIT & NTRK-like family, member 3	2.52	<0.001
ABCC9	ATP-binding cassette, subfamily C (CFTR/MRP), member 9	2.44	0.003
SLITRK6	SLIT & NTRK-like family, member 6	2.4	0.005
FAM151A	Family with sequence similarity 151, member A	2.35	0.007
CECR2	Cat eye syndrome chromosome region, candidate 2	2.34	<0.001
JAKMIP2	Janus kinase & microtubule interacting protein 2	2.32	0.013
LIPC	Lipase, hepatic	2.32	0.004
TULP3	Tubby like protein 3	2.31	0.035

Table 3 (*continued*)

Gene symbol	Gene name	Fold change	*p*
PCOLCE2	Procollagen C-endopeptidase enhancer 2	2.16	0.001
LPAL2	Lipoprotein, Lp(a)-like 2, pseudogene	2.08	0.044
LRRTM3	Leucine rich repeat transmembrane neuronal 3	2.08	<0.001
Others			
KCNN2	Potassium conductance calcium-activated channel, subfamily N, member 2	7.73	<0.001
NPY6R	Neuropeptide Y receptor Y6	4.7	0.005
CFHR3	Complement factor H-related 3	4.01	0.048
KCNJ3	Potassium inwardly-rectifying channel, subfamily J, member 3	3.15	0.013
SLC5A12	Solute carrier family 5 (sodium/glucose cotransporter), member 12	3.05	0.038
SLCO1B3	Solute carrier organic anion transporter family, member 1B3	2.63	0.001
SLC17A2	Solute carrier family 17 (sodium phosphate), member 2	2.44	0.005
SLC34A1	Solute carrier family 34 (sodium phosphate), member 1	2.44	0.005
SLC16A10	Solute carrier family 16, member 10 (aromatic amino acid transporter)	2.21	0.002

as well as cirrhotic change of the hepatic parenchyma. By contrast, three genes that are involved in regulating cellular process including cell growth, cell differentiation, mitotic cycling, embryonic development, reproduction, and tissue remodeling were downregulated, indicating that the self-renewal ability of the cirrhotic liver might be relatively limited (Table 5).

In terms of genes related to cancer, seven tumor-associated genes were significantly upregulated in the cirrhotic liver compared to the noncirrhotic liver. Of these, three genes were presented in various tumors, and four genes were associated with cancer behavior related to cancer cell invasion, angiogenic properties, and therapeutic sensitivity. Although none of these genes were directly related to primary liver tumor, these results suggested that the microenvironment contained in cirrhotic liver might be affecting oncogenesis or tumorigenesis as compared to the noncirrhotic liver (Table 6).

Significant signaling pathway and interaction network

To explore the putative signaling pathway and interaction network associated with cirrhosis liver, MetaCore and pathway analyses were further performed. Twenty-six signaling pathways were statistically significant, and a signaling pathway termed "Cell adhesion ECM_remodeling" was involved by most genes of the study. Three upregulated genes (*TIMP1*, *MMP-7*, and *Ezrin*) and one downregulated gene (*MMP-12*) were identified to participate in the Cell adhesion ECM_remodeling pathway (Figure 2), indicating cirrhotic liver might be associated with ECM remodeling, which involves the normal physiological processes of reproduction, proliferation, cell motility and adhesion, wound healing, angiogenesis, as well as disease processes. Meanwhile, the interaction network analysis resulted in 21 networks, and Figure 3 illustrates the most significant interaction network. The interaction network contained 137 genes, and 87 of them were from the current study. Additionally, the interaction network was composed of genes regarding regulation of cell proliferation (41.1%), positive regulation of gene expression (38.8%), positive regulation of transcription from RNA polymerase II promoter (32.6%), regulation of epithelial cell proliferation (21.7%), and organ development (51.9%).

Discussion

The mechanisms responsible for the initiation and process of liver regeneration are widely explored in terms of their physiological, biochemical, morphological, and molecular characteristics [1,2]. A deeper understanding of liver regeneration has been pursued for several decades, and most of the new information has been uncovered using a reproducible model of partial hepatectomy from rodents. However, the regenerative ability of the cirrhotic liver is relatively limited because of diffuse fibrosis of the hepatic parenchyma, which prohibits liver resection in patients with liver cirrhosis. Hence, it is imperative to understand the differences between a cirrhotic liver and a healthy liver. This study characterized the differences in gene expression in the cirrhotic liver using microarray technology. Furthermore, the differentially expressed genes were categorized into subgroups based on the molecular basis of their major role, which could be informative for our understanding of the pathophysiology and regeneration capacity of the cirrhotic liver.

The invention of new scientific instruments and methodologies allows for new ways of exploring medical questions. Microarray technology has become one of the most sophisticated and widely used methods for identification of differentially expressed genes [19,20]. A number of previous reports have shown that several gene profiles related to

Table 4 Differential expression genes regarding inflammation and immunologic reaction in the cirrhotic liver as compared with noncirrhotic liver.

Gene symbol	Signaling pathway category	Major function & signaling pathway of encoded protein
Upregulated genes		
ELF3	KEGG:04712	Involved in mediating vascular inflammation
NCKAP1L	KEGG:04810	Only expressed in hematopoietic cells
LGALS3BP	GO:0006968	Implicated in immune response associated with natural killer (NK) & lymphokine-activated killer (LAK) cell cytotoxicity
ITGA2	KEGG:04611	Mediates the adhesion of platelets & other cell types to the extracellular matrix
OSMR	KEGG:04060	Encodes a member of the type I cytokine receptor family
CCL20	KEGG:04060	Liver & activation-regulated chemokine
CXCL6	KEGG:04060	Chemokine (CXC motif) ligand 6 (granulocyte chemotactic protein 2)
IL32	GO:0005125	Induces the production of TNFα from macrophage cells
JAG1	KEGG:04668	Plays a role in hematopoiesis
SPP1	KEGG:04151	Upregulates expression of interferon-gamma & interleukin-12
OAS2	KEGG:05160	Involved in the innate immune response to viral infection
MX1	KEGG:02020	Environmental information processing & signal transduction
UBASH3A	GO:0050860	Facilitates growth factor withdrawal-induced apoptosis in T cells
A2M	KEGG:04610	Proteolytic cascade in blood plasma, mediator of innate immunity, & a nonspecific defense mechanism against pathogens
CD24	KEGG:04640	Expressed on mature granulocytes & in many B cells.
LGALS3	GO:0070663	Plays a role in numerous cellular functions including innate immunity, cell adhesion & T cell regulation
HLA-A	KEGG:04650	Natural killer cell mediated cytotoxicity
RASSF5	KEGG:04015	Regulates lymphocyte adhesion & suppresses cell growth in response to activated Rap1 or Ras
Downregulated genes		
IL1RAP	KEGG:04060	Initiates signaling events that result in the activation of interleukin 1-responsive genes

GO = Gene Ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes.

cirrhotic liver were associated with other underlying liver disease [20—22], and many of those genes in terms of similar categories and biologic functions were noted in this study as well. Importantly, some genes related to immune response and cytokines were identified. *OSMR*, *CCL20*, *CXCL6*, *OAS2*, and *IL32* were upregulated in cirrhosis, whereas *IL1RAP* was downregulated in cirrhotic liver. Additionally, these cytokines were identified to participate in a common cytokine—cytokine receptor interaction pathway in this study. The results suggested that an increased expression of immune responsive genes and cytokines were associated with fibrosis progress. Meanwhile, specific cytokines such as OAS2 might be also connected with viral infection, which is in agreement with previous evidence that involved the innate immune response to viral infection [23].

Numerous animal studies have examined the expression of genes related to regeneration following partial hepatectomy [24—27]. Studies also showed evidence illustrating the shift in metabolic function and energy balance in regenerating livers of rodents [28—30]. However, the majority of experiments looked at animals with healthy liver parenchyma. In the clinical setting, patients with liver disease associated with various degrees of cirrhosis are very common. This study analyzed cirrhotic livers from humans, so the results may be more transferrable to a clinical setting. Genes related to various cellular functions in terms of cell differentiation, division, growth regulation, wound healing, and apoptosis showed different expression patterns in cirrhotic liver. Additionally, few genes related to the ECM remodeling pathway were significant in cirrhotic liver, which is consistent with previous reports that showed a connection between liver cirrhosis and process of apoptosis, cell repair, wound healing, and cell proliferation [12,13,31].

Although innate immunity has been described to be important for liver regeneration [32—34], few genes involved in liver regeneration were differentially expressed in the cirrhotic liver of this study. Meanwhile, a number of genes associated with metabolic liver functions such as bile acid metabolism and protein metabolism were markedly decreased in the cirrhotic liver compared to the healthy liver. Differential expression of genes encoding enzymes that play roles in glucose metabolism, lipid metabolism, bile secretion, and hormone metabolism was also observed.

However, this study is limited by its small sample size and heterogeneous patient characteristics, and we are not able to determine what extent and degree of liver cirrhosis would affect differential gene expression in this study.

Table 5 Differential expression genes involved in cell differentiation, division, growth regulation, wound healing, and apoptosis in the cirrhotic liver.

Gene symbol	Signaling pathway category	Major function & signaling pathway of encoded protein
Upregulated genes		
GDF15	GO:0048869	Regulates tissue differentiation & maintenance
CHEK2	KEGG:04110	A cell cycle checkpoint regulator & putative tumor suppressor
HTRA1	GO:0050678	Regulates the availability of insulin-like growth factors (IGFs) by cleaving IGF-binding proteins
MMP7	KEGG:04310	Involved in wound healing, regulates the activity of defensive in intestinal mucosa
APOBEC3C	GO:0040029	Has roles in growth or cell cycle control
CHST9	KEGG:00513	Critical for cell—cell interaction, signal transduction, & embryonic development
LIPH	KEGG:01100	A lipid mediator that stimulates cell proliferation & motility
TGM2	KEGG:04210	Appears to be involved in apoptosis
EZR	GO:0032989	Plays a key role in cell surface structure adhesion, migration, & organization
FAT1	GO:0009653	Important in developmental processes & cell communication
GPC3	KEGG:05205	Plays a role in the control of cell division & growth regulation
IFI6	GO:2001233	Plays a critical role in the regulation of apoptosis
CDH6	GO:0048729	Plays critical roles in cell differentiation & morphogenesis
LOXL4	GO:0071840	Essential to the biogenesis of connective tissue
LUM	GO:0061448	Regulate collagen fibril organization & circumferential growth, corneal transparency, & epithelial cell migration & tissue repair
MVP	GO:0031099	Plays a role in multiple cellular processes by regulating the MAP kinase, JAK/STAT, & phosphoinositide 3-kinase/Akt signaling pathways
TSPAN8	GO:0007166	Plays a role in the regulation of cell development, activation, growth, & motility
RASD1	KEGG:04713	Has a role in dexamethasone-induced alterations in cell morphology, growth, & cell—extracellular matrix interactions
SORT1	KEGG:04142	The encoded protein binds a number of unrelated ligands that participate in a wide range of cellular processes
Downregulated genes		
PTPRD	GO:0022603	Regulation of anatomical structure & morphogenesis
PTPRS	GO:0009888	Regulates a variety of cellular processes including cell growth, differentiation, mitotic cycle, & oncogenic transformation
MMP12	GO:0032502	Involved in embryonic development, reproduction, & tissue remodeling, as well as in disease processes

GO = Gene Ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes.

Table 6 Differential expression of tumor associated gene in the cirrhotic liver.

Gene symbol	Signaling pathway category	Major function & signaling pathway of encoded protein
Upregulated genes		
TMPRSS3	GO:0019538	A gene overexpression in ovarian cancer
TMPRSS4	KEGG:05164	A gene overexpressed in pancreatic carcinoma
ENPP2	KEGG:00565	Stimulates the motility of tumor cells & has angiogenic properties
TUSC3	KEGG:01100	Detected in many epithelial tumor cell lines
TYMS	KEGG:00240	A target for cancer chemotherapeutic agents
CDHR2	GO:0030308	Represents a new candidate for tumor suppression
TTC9	GO:0044237	May play a role in cancer cell invasion & metastasis
Downregulated genes		
STEAP1	KEGG:04978	Upregulated in multiple cancer cell lines

GO = Gene Ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes.

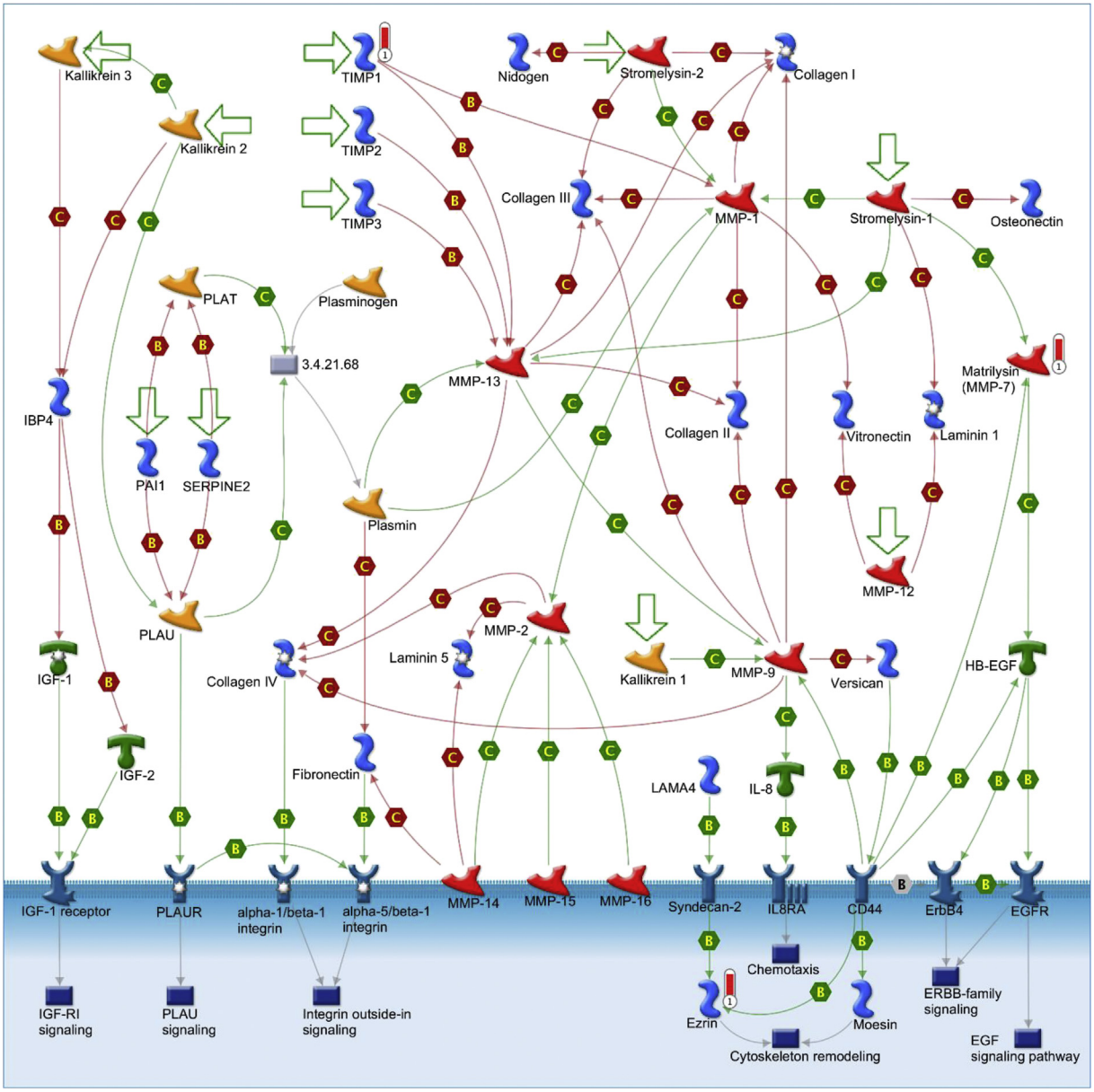

Figure 2. Illustration of signaling pathway "Cell adhesion ECM_remodeling." Four genes including three upregulated genes (*TIMP1*, *MMP-7*, and *Ezrin*) and one downregulated gene (*MMP-12*) from the current study were identified to participate in the pathway.

Additionally, the transition from chronic liver disease to cirrhosis involves a lengthy process that includes inflammation, angiogenesis, and fibrogenesis. Each of the processes at different time points might express various cascades of genes, and the real situation of gene expression in cirrhosis may not be adequately reflected by a randomly selected time point as in the current study. Apart from that, most genes usually act as part of diverse signaling pathway, and multiple genes interacting through a signaling pathway on cellular function is more significant than that of a single gene. Therefore, further work with more patients and solid study might be necessary to determine the actual contribution of each element.

Taken together, our results showed that the cirrhotic liver has a unique environment compared to the noncirrhotic liver. Specifically, the cirrhotic liver has strong immune responses including inflammation and immunological reaction, which could be a consequence of liver fibrosis. Moreover, the cirrhotic liver is in an unstable state, in which hepatocyte damage, growth regulation, wound healing, and apoptosis are constantly occurring. This would lead to scar formation as well as cirrhotic change of the hepatic parenchyma. Although generalizations about the study cannot be easily made, several remarkable exploration might be helpful in future research as well as provide information that could be used to better understand liver cirrhosis.

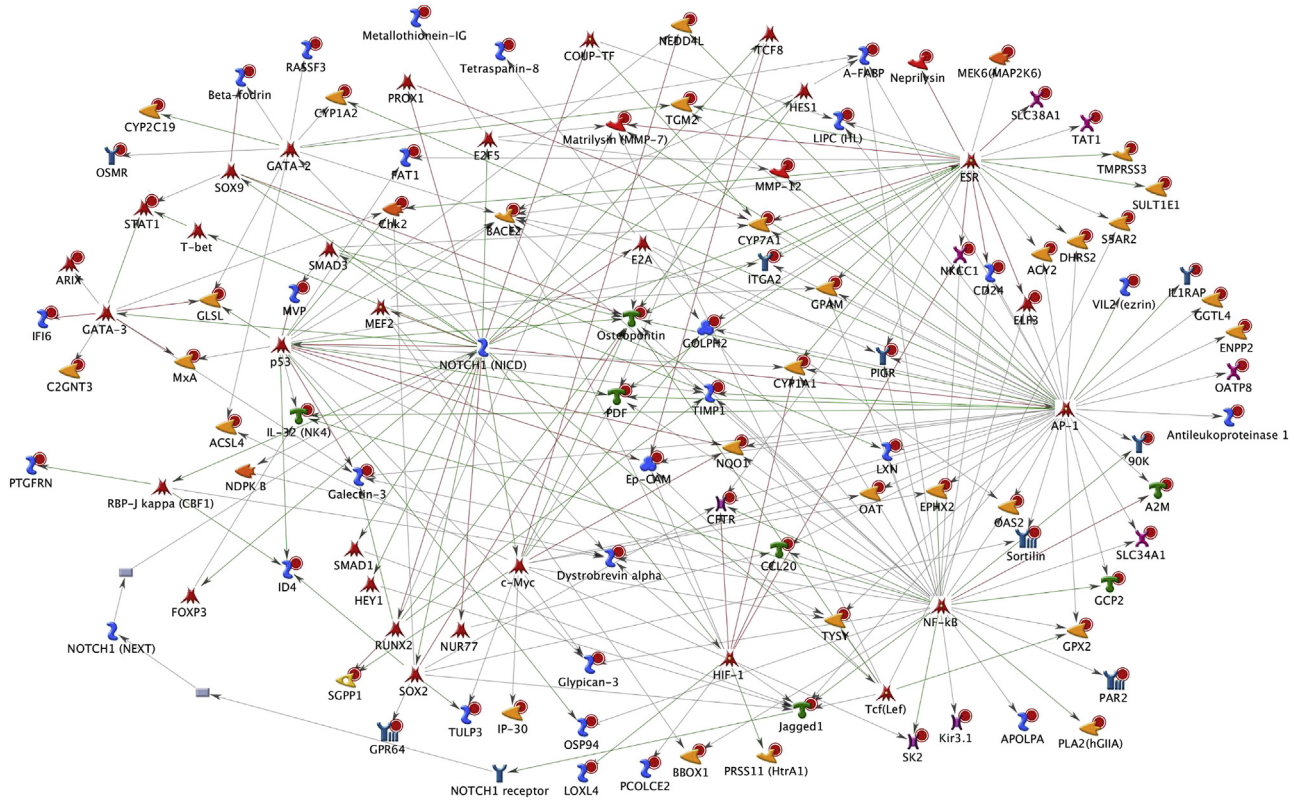

Figure 3. Significant interaction network analyzed from MetaCore. The interaction network comprised 137 genes that contained 87 significant genes (red circle) of the study.

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