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Gene Regulations in HBV-Related Liver Cirrhosis Closely Correlate with Disease Severity

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Liver cirrhosis (LC) is defined as comprising diffuse fibrosis and regenerating nodules of the liver. The biochemical and anatomical dysfunction in LC results from both reduced liver cell number and portal vascular derangement. Although several studies have investigated dysregulated genes in cirrhotic nodules, little is known about the genes implicated in the pathophysiologic change of LC or about their relationship with the degree of decompensation. Here, we applied cDNA microarray analysis using 38 HBsAg-positive LC specimens to identify the genes dysregulated in HBV-associated LC and to evaluate their relation to disease severity. Among 1063 known cancer- and apoptosis-related genes, we identified 104 genes that were significantly up- (44) or down- (60) regulated in LC. Interestingly, this subset of 104 genes was characteristically correlated with the degree of decompensation, called the Pugh-Child classification (20 Pugh-Child A, 10 Pugh-Child B, and 8 Pugh-Child C). Patient samples from Pugh-Child C exhibited a distinct pattern of gene expression relative to those of Pugh-Child A and B. Especially in Pugh-Child C, genes encoding hepatic proteins and metabolizing enzymes were significantly down-regulated, while genes encoding various molecules related to cell replication were up-regulated. Our results suggest that subsets of genes in liver cells correspond to the pathophysiologic change of LC according to disease severity and possibly to hepatocarcinogenesis.

Keywords: cDNA microarray, Hepatitis B virus (HBV), Hepatocellular carcinoma (HCC), Liver cirrhosis (LC), Pugh-child classification

Abbreviations: LC, HCC, HBV, HBsAg, HBeAg

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Introduction

Liver cirrhosis (LC) is a leading cause of death and hepatocellular carcinoma (HCC) in the world's endemic areas of hepatitis B virus (HBV) infection (Liaw *et al.*, 1989; Montalto *et al.*, 2002). Usually, LC is anatomically defined as diffuse fibrosis and regenerating nodules of liver, and patients die from various complications associated with cirrhotic decompensation (Friedman, 2003) or HCC with an annual incidence of between 2.5% and 7% of cirrhotic liver cases (Montalto *et al.*, 2002). The degree of cirrhotic decompensation is usually stratified with the Pugh-Child classification, which is composed of albumin, bilirubin, prothrombin time, ascites and encephalopathy (Pugh *et al.*, 1973). It has been generally recognized that biochemical and anatomical dysfunction in LC results from both reduced liver cell number and portal vascular derangement due to chronic, destructive, sclerosing, and inflammatory injury of the liver (Friedman, 2003), in which various cytokines, inflammatory cells and hepatocytes orchestrate the inflammatory response, while hepatic stellate cells, myofibroblasts and matrix molecules participate in fibrogenesis (Olaso and Friedman, 1998; Friedman, 2003). Reportedly, aberrant loss of heterozygosity of DNA is frequent in cirrhotic liver nodules (Roncalli *et al.*, 2000; Nagai *et al.*, 2004), suggesting that genetic alterations of hepatocytes may also be involved in the LC pathogenesis. Although a few studies have investigated dysregulated genes in cirrhotic nodules, little is known about the genes implicated in the pathophysiologic change of LC, or about their relationship with the degree of decompensation.

To understand gene dysregulation in liver diseases, the complementary DNA (cDNA) microarray technology has been used for global gene expression analysis of hepatitis viruses (Otsuka *et al.*, 2003), chronic viral hepatitis (Honda *et al.*, 2001), cirrhotic nodule (Nagai *et al.*, 2004), dysplastic nodules (Anders *et al.*, 2003), and HCC (Okabe *et al.*, 2001; Xu *et al.*, 2001). Previously, we applied the cDNA microarray analysis of 55 liver tissues from LC and HCC patients and found the different expression profiles in LC, HCC and

normal liver tissues (Kim and Park, 2005). Especially, 25 cirrhosis-specific genes could be used to divide the LC samples into inflammatory active cirrhosis and inflammatory inactive cirrhosis (Kim and Park, 2005).

Here, we applied the cDNA microarray analysis using 38 HBsAg-positive LC specimens to identify the subset of genes closely associated with HBV-related LC and to evaluate their relation to the disease severity according to Pugh-Child classification. Our data would provide an insight into the pathophysiologic mechanism of cirrhotic decompensation at the molecular level.

Materials and Methods

Tissue samples. Thirty-eight tissue specimens of cirrhosis were obtained from HBsAg-positive LC patients (mean age 51 ± 9 years; 33 males and 5 females; 15 HBeAg-positive and 23 HBeAg-negative) during surgical resection of HCC or liver transplantation. They consisted of 20 Pugh-Child A, 10 B, and 8 C (Table 1). As controls, 6 normal liver tissues were obtained from non-B, non-C patients with other malignant diseases during partial hepatectomy. Informed consent was obtained from each patient for operation and tissue utilization. Every sample collection was performed from Bundang Jesaeng General Hospital, Korea in accordance with the hospital guideline for tissue banking. The tissue was snap-frozen within 30min of harvesting and stored in a nitrogen tank until used.

cDNA microarray experiment. Total RNA preparation and cDNA microarray (containing 1063 genes) experiments were performed described in Kim *et al.* (Kim and Park, 2005; Kim *et al.*, 2006).

cDNA microarray analysis. The cDNA microarray results were analyzed using GeneSpring version 5.0 (Silicon Genetics). The overall intensities were normalized with a correction coefficient obtained using the negative controls. For data normalization, the intensities of all spots obtained from hybridization were averaged and the intensity of each corrected DNA spot was adjusted as the average intensity ratio (Cy5/Cy3 = 1.0). A difference of more than 2.0 or less than 0.5 in the median of the raw signal to control signal ratio was defined to be up- or down-regulated, respectively. The expression ratio, raw-to-control signal ratio, was expressed as mean ± SD. Differences in the frequency between groups were analyzed by ANOVA. Differences in the expression levels between groups were analyzed using the *t*-test. Differences with *P* values of less than .05 were considered significant.

Quantitative real time RT-PCR analysis. For RT-PCR confirmation of the microarray results, total RNA was extracted from 29 blinded samples and reverse transcribed with oligo dT primers and the Thermoscript kit. Quantitative real time PCR analysis was performed as previously described (Kim *et al.*, 2003; Lee *et al.*, 2007). In brief, the templates and primer sets were mixed with 2x QuantiTect SYBR Green PCR Master Mix, and 20 cycles of PCR were performed using a Rotor-Gene real-time PCR machine. All experiments were performed at least twice.

Results

Identification of significantly dysregulated genes in HBV-associated LC. A total of 104 genes, comprising 44 up- and 60 down- regulated, were significantly dysregulated in HBV-associated LC. By statistical analysis, the mean intensity of expression level of each gene calculated by log ratio was correlated with the degree of cirrhotic decompensation

Table 1. Clinical profiles of LC grouped by Pugh-Child classification

Item	Pugh-Child A (N = 20)	Pugh-Child B (N = 10)	Pugh-Child C (N = 8)
Age (years old)	53 ± 11	50 ± 6	51 ± 9
Sex (M/F)	18/2	10/0	5/3
HBeAg(+)/(-)	7/13	4/6	4/4
Accompanied by HCC	17 (85%)	6 (60%)	2 (26%)
AFP (ng/dL)	505 ± 850	388 ± 671	415 ± 876
Platelet (/mm3)*	115,000 ± 74,000	83,000 ± 32,000	51,000 ± 25,000
Albumin (gr/dL)*	3.6 ± 0.4	3.0 ± 0.5	2.9 ± 0.4
Total bilirubin (mg/dL)*	0.9 ± 0.3	1.8 ± 1.8	12.0 ± 10.9
Prothrombin time (%)*	90 ± 19	71 ± 18	31 ± 15
AST (IU/L)	66 ± 36	57 ± 33	79 ± 41
ALT (IU/L)	62 ± 66	45 ± 24	90 ± 108
ALP (IU/L)	241 ± 78	213 ± 48	204 ± 69
GGT (IU/L)	89 ± 74	71 ± 97	31 ± 36
Total cholesterol (mg/dL)*	153 ± 36	134 ± 43	65 ± 18
Triglyceride (mg/dL)*	83 ± 40	80 ± 46	35 ± 38
RBP (gr/dL)*	2.3 ± 1.3	2.7 ± 2.8	1.1 ± 0.2

*Parameters which have significant differences among 3 groups ($p < 0.05$).

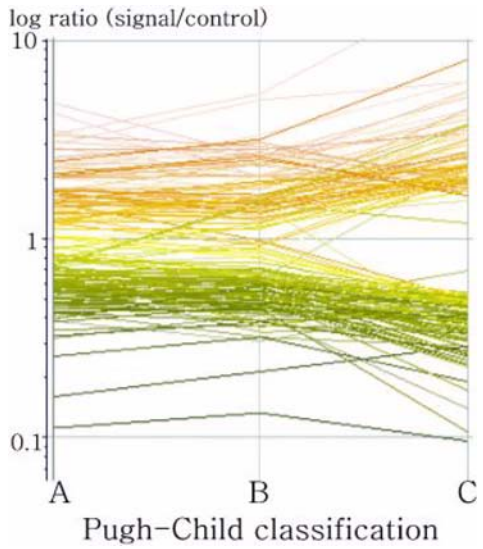

Fig. 1. Comparison of the mean intensity of the expression level (calculated by log ratio) between 104 up- and down-regulated genes, which were significantly changed in LC; Pugh-Child A, B and C. This figure strongly suggests that dysregulated genes in HBV-associated LC are closely correlated with the degree of decompensation according to the Pugh-Child classification.

according to the Pugh-Child classification (Fig. 1).

The top 5 ranked genes among the 44 up-regulated genes were CDH3, RCN2, RPS4Y, CDK4 and CPE in the following order of frequency; 31 (81.2%), 29 (76.3%), 29 (76.3%), 27 (71.1%) and 28 (73.7%) of 38 LC specimens, respectively. The top 5 down-regulated genes were PZP, CHAF1A, FCGRT, MMP10 and NQO1 in the following order of frequency; 36 (94.7%), 33 (91.7%), 29 (76.3%), 28 (73.7%) and 26 (68.4%) of 38 LC specimens, respectively.

Groups of genes differentially expressed in LC according to Pugh-Child classification. As shown in Fig. 1, many genes were correlated with the Pugh-Child classification and a distinct pattern was especially showed in Pugh-Child C. We compared the prevalence of 104 dysregulated genes in relation to the degree of decompensation according to the Pugh-Child classification. Based on the biological function from the functional annotations described in the GeneBank and the corresponding references, we categorized the 104 genes into several groups, as summarized in Table 2 (For more complete lists, see Supplementary Tables 2-7).

Genes encoding hepatic proteins and hepatic metabolizing enzymes such as C6, and CYP3A4 were significantly down-regulated in Pugh-Child C, as shown in Table 2. A gene encoding hepatic enzyme, GSTT1, was up-regulated in half of Pugh-Child A, but in none of Pugh-Child C. The pregnancy-zone protein (PZP), one of the alpha(2)M gene family of proteins, shown in Table 2, has been reported to increases in serum during late pregnancy, but, frequently decreases in gynecological tumors (Teng *et al.*, 1994). In our study, PZP was markedly down-regulated in most (94.7%) of LC, suggesting that PZP may be an early marker of LC.

Table 2 and Supplementary Table 3 list various genes encoding proteins involved in signal transduction, cell cycle, cell proliferation, liver growth factor and liver cell death. Two of genes encoding proteins associated with signal transduction - YES1 and CBLB, - were significantly up-regulated in Pugh-Child C, compared to those of Pugh-Child A. Genes encoding molecules associated with cell cycle and cell proliferation, such as CDK4, and MAPK12, were significantly up-regulated in Pugh-Child C, compared to those of Pugh-Child A. CLK2 was down-regulated in 31.6% (12/38) of LC, but more frequently in Pugh-Child C. Genes encoding liver growth factors - HDGF and SOCS2 - were down-regulated in LC, while pleiotrophin (PTN) was up-regulated. Among these, the expression of HDGF was undetectable in 63% of Pugh-Child C and SOCS2 was more significantly changed in Pugh-Child C than in Pugh-Child A and B. Regarding genes encoding cell death factors, CASP10 and BAD were more frequently up-regulated in Pugh-Child C than in Pugh-Child A.

Table 2 and Supplementary Table 4 show the genes encoding nuclear proteins. RBM4, and CEBPG were more frequently up-regulated in Pugh-Child C than in Pugh-Child A and B. CHAF1A, E2F5 and DCTD were frequently down-regulated in LC; 86.8, 63.2, and 52.6%, respectively. Genes encoding various proteins associated with cell contact, extracellular matrix (ECM) and cytoskeleton were significantly dysregulated in LC, as shown in Table 2 and Supplementary table 5. CDH3 was frequently up-regulated in 81.6% (31/38) of LC specimens, regardless of disease severity, but its expression intensity was frequently higher in Pugh-Child C than in Pugh-Child A and B (9.6 ± 6.0 fold vs. 3.1 ± 2.2 fold and 4.4 ± 3.0 fold, respectively). GALIG was also frequently up-regulated in Pugh-Child C. JUP was down-regulated in accordance with Pugh-Child classification. Among the genes encoding ECM molecules, decorin (DCN) was more frequently changed in Pugh-Child C than in Pugh-Child A and B, while MMP10 and HYAL1 were frequently down-regulated. A gene encoding cytoskeleton, DNM2, was frequently down-regulated in Pugh-Child C.

In Table 2 and Supplementary Table 6, two genes encoding transporter proteins, ATP5B and TCIRG1, were more frequently up-regulated in Pugh-Child C than in Pugh-Child A and B. Three genes encoding solute carrier family proteins, SLC10A, SLC11A2, and SLC22A1, were more frequently down-regulated in Pugh-Child C than those of Pugh-Child A and B. Two genes encoding proteins with calcium binding property in endoplasmic reticulum were identified; RCN2 was frequently up-regulated in LC, while CLGN was down-regulated. Two genes encoding plasma membrane-associated proteins, STOM and CXX1, were found to be more frequently up-regulated in Pugh-Child C than in Pugh-Child A and B. Table 2 and Supplementary Table 7 show the up- or down-regulated genes encoding immune response-regulating proteins in LC. Especially, HLA-C and IL15RA were more frequently up-

Table 2. Genes up or down regulated by Pugh-Child classification

Gene Bank Access #	Gene Names [Gene Symbol]	Child A (N = 20)		Child B (N = 10)		Child C (N = 8)	
	Up-regulated genes encoding hepatic proteins or enzymes						
H99813	glutathione S-transferase theta 1 [GSTT1]	10	50%	3	30%	0	0%
	Down-regulated genes encoding hepatic proteins or enzymes						
AA905669	pregnancy-zone protein precursor [PZP]	19	95%	9	90%	8	100%
N59396	complement C6 precursor [C6]	8	40%	4	40%	8	100%
T71349	cytochrome P450IIIA4 [CYP3A4]	7	35%	4	40%	8	100%
	Up-regulated genes encoding signal transduction and oncogenes						
N36882	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1 [YES1]	7	35%	7	70%	8	100%
AA704729	Cas-Br-M (murine) ecotropic retroviral transforming sequence b[CBLB]	6	30%	1	10%	7	88%
	Down-regulated genes encoding signal transduction and oncogenes						
H84871	serine threonine kinase 39 [STK39]	8	40%	0	0%	6	75%
	Up-regulated genes encoding proteins associated with cell cycle and cell proliferation						
AA486208	cyclin-dependent kinase 4 [CDK4]	12	60%	8	80%	7	88%
AI936909	mitogen-activated protein kinase 12 [MAPK12]	7	35%	6	60%	8	100%
	Down-regulated genes encoding proteins associated with cell cycle and cell proliferation						
AA282845	cell division cycle(CDC)-like kinase 2 [CLK2]	4	20%	3	30%	5	63%
	Up-regulated genes encoding liver growth factors						
AA001449	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1) [PTN]	8	40%	2	20%	7	88%
	Down-regulated genes encoding liver growth factors						
AA453749	hepatoma-derived growth factor (high-mobility group protein 1-like) [HDGF]	8	40%	4	40%	5	63%
AA137031	STAT induced STAT inhibitor-2 [SOCS2]	3	15%	3	30%	5	63%
	Up-regulated genes encoding proteins involved in cell death						
H80712	caspase 10 [CASP10]	7	35%	1	10%	6	75%
AA460291	BCL2-antagonist of cell death [BAD]	6	30%	3	30%	6	75%
	Down-regulated genes encoding proteins involved in cell death						
T95052	caspase 1 [CASP1]	9	45%	7	70%	7	88%
	Up-regulated genes encoding nuclear factors						
AA454656	RNA binding motif protein 4 [RBM4]	5	25%	2	20%	7	88%
AA676804	CCAAT/enhancer binding protein [CEBPG]	3	15%	1	10%	7	88%
T69468	40S ribosomal protein S4, Y isoform [RPS4Y]	16	80%	8	80%	5	63%
	Down-regulated genes encoding nuclear factors						
AA704459	chromatin assembly factor-I p150 subunit [CHAF1A]	16	80%	9	90%	8	100%
AA455521	E2F transcription factor 5 [E2F5]	12	60%	4	40%	8	100%
AA448207	dCMP deaminase [DCTD]	9	45%	5	50%	6	75%
	Up-regulated genes encoding proteins involved in cell contact						
AA425217	cadherin 3, type 1, P-cadherin [CDH3]	15	75%	8	80%	8	100%
AA630328	galectin-3 internal gene [GALIG]	8	40%	6	60%	7	88%
	Down-regulated genes encoding proteins involved in cell contact						
AA035637	junction plakoglobin [JUP]	6	30%	6	60%	8	100%
	Up-regulated genes encoding extracellular matrix proteins						
H64138	decorin [DCN]	0	0%	2	20%	5	63%
	Down-regulated genes genes encoding extracellular matrix proteins						
AA857496	matrix metalloproteinase 10 (stromelysin-2) [MMP10]	14	70%	7	70%	7	88%
AA464196	hyaluronoglucosaminidase 1 [HYAL1]	1	5%	2	20%	4	50%

Table 2. Continued

Gene Bank Access #	Gene Names [Gene Symbol]	Child A (N = 20)		Child B (N = 10)		Child C (N = 8)	
	Up-regulated genes encoding cytoskeleton proteins						
AA180013	nebulin-related anchoring protein [NRAP]	7	35%	1	10%	5	63%
	Down-regulated genes encoding cytoskeleton proteins						
AA780897	dynamin 2 [DNM2]	3	15%	3	30%	8	100%
	Up-regulated genes encoding transporter molecules						
AA708298	ATP synthase, H+ transporting [ATP5B]	9	45%	6	60%	8	100%
AI359884	T-cell, immune regulator 1 [TCIRG1]	4	20%	2	20%	6	75%
	Down-regulated genes encoding transporter molecules						
T68568	solute carrier family 10 [SLC10A1]	4	20%	3	30%	8	100%
AA702013	solute carrier family 22 [SLC22A1]	1	5%	1	10%	5	63%
	Up-regulated genes encoding calcium-binding proteins						
AA598676	reticulocalbin 2, EF-hand calcium binding domain [RCN2]	15	75%	6	60%	8	100%
	Down-regulated genes encoding calcium-binding proteins						
AA778675	calmegin [CLGN]	9	45%	6	60%	5	63%
	Genes encoding cell membrane-associated proteins Up-regulated genes						
R62817	stomatin [STOM]	3	15%	2	20%	7	88%
W72596	CAAX box 1 [CXX1]	3	15%	2	20%	7	88%
	Up-regulated genes encoding proteins associated with immune regulation						
AA464246	HLA class I histocompatibility antigen [HLA-C]	7	35%	3	30%	8	100%
AA054754	interleukin 15 receptor alpha [IL15RA]	3	15%	5	50%	8	100%
AA427664	TIA1 cytotoxic granule-associated RNA binding protein [TIA1]	12	60%	7	70%	5	63%
	Down-regulated genes encoding proteins associated with immune regulation						
AA256132	interleukin 1 receptor accessory protein [IL1RAP]	12	60%	7	70%	8	100%
H70491	C-type lectin-like receptor-2 [CLEC-2]	5	25%	3	30%	6	75%

regulated in Pugh-Child C than in Pugh-Child A and B, whereas CLEC-2 was more frequently down-regulated in Pugh-Child C.

To validate the gene expression shown in Table 2, we examined the expressions of 10 of these genes in same samples used for microarray (20 Child A, 10 Child B and 8 Child C) using quantitative real time RT-PCR analysis. The levels of gene expression were measured relative to three normal mixed samples (Kim and Kim, 2003; Suh *et al.*, 2006), and the average gene expression levels were calculated for the groups of each tissue (Table 3). Consistent with the microarray results (Table 2), the up-regulated genes - CBLB, CASP10, DCN, and CXX1 were up-regulated in Pugh-Child C samples, while CYP3A4, E2F5, DMN2, and SLC10A were down-regulated in in Pugh-Child C samples. In our study, CDK9 was up-regulated and PZP was markedly down-regulated in Pugh-Child A, B and C.

Discussion

Our analysis demonstrated that genetic alteration of cirrhotic liver is remarkable in Pugh-Child C, relative to Pugh-Child A and B. Especially, the genes encoding hepatic proteins, hepatic enzymes and metabolizing enzymes, as listed in Table 2, were significantly down-regulated in Pugh-Child C. These results suggest that, in addition to reduced liver cell mass and deranged anatomical structure of liver, the inhibition of a subset of genes, which are normally expressed in properly functioning hepatocytes, may be responsible for hepatic functional failure in decompensated cirrhosis.

With regard to liver regenerating activity in LC, our study revealed that various genes encoding a wide variety of molecules involved in signal transduction, cell cycle, cell proliferation, liver growth factors and nuclear factors were markedly dysregulated in accordance with disease severity. Based on gene up- and down-regulated functions, it is possible that, despite the suppression of hepatic growth factors, internal mechanisms for liver cell replication are activated in LC and apoptotic cell death mechanism may play a role in the loss of hepatocytes in decompensated cirrhosis.

Three genes encoding nuclear factors, RBM4, CEBPG and WT1, were significantly up-regulated in Pugh-Child C, suggesting that some genes encoding nuclear factors are actively responding to cirrhotic liver injury. Another three nuclear factor genes, CHAF1A, E2F5 and DCTD, were

Table 3. Real time PCR analysis for subset of genes up or down regulated by Pugh-Child classification

	Normalized Average Gene Expression Levels[1]		
	Child A (20^+)	Child B (10^+)	Child C (8^+)
AA704729 CBLB	1.79 ± 0.91	1.53 ± 0.32	2.03 ± 0.41
AA486208 CDK9	3.24 ± 1.55	3.68 ± 3.00	3.72 ± 1.43
H80712 CASP10	1.86 ± 0.91	1.39 ± 0.37	2.54 ± 1.07
H64138 DCN	1.19 ± 0.37	1.32 ± 0.71	2.29 ± 0.94
W72596 CXX1	1.62 ± 0.54	1.48 ± 0.54	2.26 ± 0.55
AA905669 PZP	0.15 ± 0.14	0.18 ± 0.17	0.10 ± 0.03
T71349 CYP3A4	0.67 ± 0.38	0.62 ± 0.43	0.28 ± 0.14
AA455521 E2F5	0.51 ± 0.29	0.54 ± 0.26	0.16 ± 0.12
AA780897 DMN2	0.81 ± 0.42	0.71 ± 0.53	0.30 ± 0.14
T68568 SLC10A	0.66 ± 0.27	0.57 ± 0.28	0.26 ± 0.14

1: Quantitative real time RT-PCR was used to assess the RNA copy numbers from samples, normalized against the average copy numbers from the 3 normal samples; >1 indicates up-regulation, <1 indicates down-regulation and ~1 indicates no change in expression compared to the normal samples.
+: The numbers indicate the numbers of each samples used for quantitative real time RT-PCR analysis. The patients used in this study were same from those used in the microarray analysis.

significantly down-regulated in LC, regardless of disease severity, suggesting that the molecular profile of cirrhotic liver for DNA synthesis may be similar to the late phase of normal liver regeneration, in which DNA synthesis is suppressed (Fukuhara *et al.*, 2003).

Table 2 and Supplementary Table 5 indicate that P-cadherin, galectin-3 and plakoglobin may play important roles in both LC pathogenesis and hepatocarcinogenesis. ECM-associated factors play an essential role in liver injury associated with tissue remodeling. MMP10 was frequently down-regulated, regardless of the Pugh-Child classification. DCN was frequently up-regulated in Pugh-Child C, while HYAL1 was frequently down-regulated. Taken altogether, it is suggested that fibrogenesis in LC is actively regulated by the orchestration of a subset of genes encoding ECM.

We found that genes encoding cytoskeleton and transporter were frequently dysregulated in LC (Table 2). Since various solute exchanges are mediated by cytoskeletons and transporters, which play important signaling roles for internalization of membranes and stimulus-secretion coupling, it is suggested that a subset of genes encoding cytoskeletons and transporter molecules actively responds to the pathophysiologic change of LC. In addition, the subset of intracellular proteins RCN2, CLGN, STOM and CXX1 may be connected to the pathophysiologic changes occruing in the cirrhotic liver.

Various immune response-regulating molecules influence on HBV-associated liver injury. HLA-C and IL15RA are augmented in response to environmental/stress stimuli and infectious agents (Perera, 2000). CLEC-2 is homologous to the NK cell receptor CD94 and to the oxidized low-density lipoprotein receptor 1, and is preferentially transcribed in the liver (Colonna *et al.*, 2000). In this study, HLA-C, IL15RA and CD5 were frequently up-regulated in Pugh-Child C, while CLEC-2 was frequently down-regulated. Regardless of the Pugh-Child classification, TIA1 was frequently up-regulated, while IL1RAP and TRAF2 were frequently down-regulated (Table 2 and Suppleemntary Table 7). Although their exact roles in HBV-associated LC remain to be elucidated, our results suggest that the orchestration of those molecules plays an important role in cirrhotic liver injury.

In summary, we found that a subset of dysregulated genes in HBV-associated LC is closely correlated with the degree of hepatic decompensation by the Pugh-Child classification. Dysregulation of particular genes in liver cells may account for the functional failure evident in decompensated cirrhosis. It is noteworthy that various genes known to be associated with oncogenesis are frequently dysregulated in cirrhotic liver, suggesting that they play in promoting hepatocarcinogenesis as well as liver cell regeneration. Therefore, our results provide an insight into the pathophysiologic changes of LC at the molecular level.

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Supplementary Table 1. Genes up-regulated in LC but not expressed in normal liver and genes down-regulated in LC

Gene Bank Access #	Gene Names [Gene Symbol]	**Child A** (N = 20)	**Child B** (N = 10)	**Child C** (N = 8)
	Genes up-regulated in LC but not expressed in normal liver (Mean ± S.D. fold)			
AA598676	reticulocalbin 2, EF-hand calcium binding domain [RCN2]	6.8 ± 8.0	4.2 ± 3.8	6.5 ± 5.7
AA708298	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide [ATP5B]	3.8 ± 4.0	3.0 ± 2.3	6.1 ± 3.1
AA994760	Pro-(alpha)3(V) collagen [COL5A3]	7.3 ± 7.5	4.5 ± 4.9	5.0 ± 1.6
AA406028	CD5 antigen (p56-62) [CD5]	1.8 ± 0.8	1.5 ± 0.9	4.9 ± 4.1
AA454146	cyclin H [CCNH]	2.1 ± 1.5	1.4 ± 0.4	4.4 ± 4.3
AA633818	fatty-acid-Coenzyme A ligase, long-chain 4 [FACL4]	2.0 ± 1.5	3.9 ± 6.7	4.2 ± 2.4
N36882	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1 [YES1]	5.8 ± 6.2	7.9 ± 6.0	31.6 ± 32.5
AA877595	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4) [CDKN2A]	2.0 ± 1.4	2.0 ± 1.3	3.5 ± 1.7
AA461513	carboxypeptidase E [CPE]	5.4 ± 5.3	4.9 ± 4.5	3.0 ± 1.9
	Genes nearly down-regulated in LC (Mean ± S.D. fold)			
AA453749	hepatoma-derived growth factor (high-mobility group protein 1-like) [HDGF]	0.62 ± 0.51	0.68 ± 0.33	0.18 ± 0.08
AA705308	fetuin B (histidine-rich glycoprotein precursor) [FETUB]	0.51 ± 0.33	0.64 ± 0.53	0.18 ± 0.12
AA256132	interleukin 1 receptor accessory protein [IL1RAP]	0.48 ± 0.16	0.42 ± 0.28	0.31 ± 0.10
AA702663	myosin IXB [MYO9B]	0.53 ± 0.25	0.54 ± 0.28	0.36 ± 0.15
AA436163	prostaglandin E synthase [PTGES]	0.62 ± 0.29	0.49 ± 0.20	0.42 ± 0.22

Supplementary Table 2. Genes encoding hepatic proteins or enzymes

Gene Bank Access #	Gene Names [Gene Symbol]	Child A (N = 20)		Child B (N = 10)		Child C (N = 8)	
	Up-regulated genes						
AA633818	fatty-acid-Coenzyme A ligase, long-chain 4 [FACL4]	8	40%	1	10%	6	75%
AA461513	carboxypeptidase E [CPE]	15	75%	8	80%	5	63%
AA521303	methionine adenosyltransferase II, beta [MAT2B]	9	45%	7	70%	4	50%
H99813	glutathione S-transferase theta 1 [GSTT1]	10	50%	3	30%	0	0%
	Down-regulated genes						
AA905669	pregnancy-zone protein precursor [PZP]	19	95%	9	90%	8	100%
AA458634	NAD(P)H dehydrogenase, quinone 1 [NQO1]	12	60%	6	60%	8	100%
N59396	complement C6 precursor [C6]	8	40%	4	40%	8	100%
T71349	cytochrome P450IIIA4 [CYP3A4]	7	35%	4	40%	8	100%
AA148230	HIV-1 Tat interactive protein 2, 30kDa [HTATIP2]	6	30%	5	50%	8	100%
N64422	cytochrome P450, family 39, subfamily A, polypeptide 1 [CYP39A1]	7	35%	6	60%	7	88%
N53136	cytochrome P450IIC8 [CYP2C8]	5	25%	6	60%	7	88%
R06458	lecithin-cholesterol (phosphatidylcholine-sterol) acyltransferase [LCAT]	3	15%	3	30%	7	88%
H53865	complement C8 alpha chain precursor [C8A]	2	10%	6	60%	7	88%
T72259	cytochrome P450IIA6 [CYP2A6]	10	50%	3	30%	6	75%
R64101	arginyl aminopeptidase (aminopeptidase B) [RNPEP]	6	30%	5	50%	6	75%
T62060	serine (or cysteine) proteinase inhibitor, clade C (antithrombin), member 1 [SERPINC1]	4	20%	1	10%	5	63%
T67006	glucokinase (hexokinase 4) regulatory protein [GCKR]	2	10%	4	40%	5	63%
AA699812	methionine adenosyltransferase I, alpha [MAT1A]	2	10%	2	20%	5	63%
H99813	glutathione S-transferase theta 1 [GSTT1]	5	25%	4	40%	4	50%
AA436163	prostaglandin E synthase [PTGES]	6	30%	6	60%	5	63%
AA705308	fetuin B (histidine-rich glycoprotein precursor) [FETUB]	8	40%	3	30%	7	88%
T71887	apolipoprotein C-IV [APOC4]	1	5%	3	30%	5	63%

Supplementary Table 3. Genes encoding signal transduction, cell cycle, growth factors & apoptosis

Gene Bank Access #	Gene Names [Gene Symbol]	Child A (N = 20)		Child B (N = 10)		Child C (N = 8)	
Genes encoding signal transduction and oncogenes							
	Up-regulated genes						
N36882	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1 [YES1]	7	35%	7	70%	8	100%
AA704729	Cas-Br-M (murine) ecotropic retroviral transforming sequence b[CBLB]	6	30%	1	10%	7	88%
AA664007	serine/threonine kinase 25 (STE20 homolog, yeast) [STK25]	2	10%	3	30%	7	88%
R93509	serine/threonine kinase 4 [STK4]	4	20%	1	10%	4	50%
AI206156	Cas-Br-M (murine) ecotropic retroviral transforming sequence [CBL]	4	20%	1	10%	5	63%
AI681064	inositol polyphosphate-5-phosphatase, 75kDa [INPP5B]	8	40%	3	30%	6	75%
T71976	phosphatidic acid phosphatase type 2B [PPAP2B]	5	25%	4	40%	5	63%
AA478543	A kinase (PRKA) anchor protein (gravin) 12 [AKAP12]	0	0%	1	10%	4	50%
	Down-regulated genes						
AA447730	pim-1 proto-oncogene serine/threonine-protein kinase [PIM-1]	8	40%	2	20%	6	75%
H84871	serine threonine kinase 39 (STE20/SPS1 homolog, yeast) [STK39]	8	40%	0	0%	6	75%
AI024862	protein kinase, cAMP-dependent, catalytic, gamma [PRKACG]	10	50%	5	50%	5	63%
H51117	phosphodiesterase 1B, calmodulin-dependent [PDE1B]	7	35%	1	10%	4	50%
Genes encoding proteins associated with cell cycle and cell proliferation							
	Up-regulated genes						
AA486208	cyclin-dependent kinase 4 [CDK4]	12	60%	8	80%	7	88%
AA877595	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4) [CDKN2A]	9	45%	4	40%	7	88%
AA454146	cyclin H [CCNH]	6	30%	0	0%	6	75%
AA016290	Retinoblastoma-binding protein 6 [RBBP6]	5	25%	2	20%	5	63%
AI936909	mitogen-activated protein kinase 12 [MAPK12]	7	35%	6	60%	8	100%
W61116	mitogen-activated protein kinase kinase [MAPKK]	8	40%	4	40%	4	50%
	Down-regulated genes						
AA282845	cell division cycle(CDC)-like kinase 2 [CLK2]	4	20%	3	30%	5	63%
Genes encoding liver growth factors							
	Up-regulated genes						
AA001449	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1) [PTN]	8	40%	2	20%	7	88%
	Down-regulated genes						
H22653	glia maturation factor, beta [GMFB]	6	30%	2	20%	7	88%
AA233738	transforming growth factor, beta 2 [TGFB2]	11	55%	7	70%	6	75%
R55303	low affinity nerve growth factor receptor (TNFR superfamily, member 16) [NGFR]	8	40%	2	20%	6	75%
AA453749	hepatoma-derived growth factor (high-mobility group protein 1-like) [HDGF]	8	40%	4	40%	5	63%
AA137031	STAT induced STAT inhibitor-2 [SOCS2]	3	15%	3	30%	5	63%
Genes encoding proteins involved in cell death (apoptosis)							
	Up-regulated genes						
H80712	caspase 10 [CASP10]	7	35%	1	10%	6	75%
AA460291	BCL2-antagonist of cell death [BAD]	6	30%	3	30%	6	75%
W02761	tumor necrosis factor receptor superfamily, member 1A [TNFRSF1A]	9	45%	7	70%	3	38%
	Down-regulated genes						
T95052	caspase 1 (interleukin-1beta converting enzyme, ICE) [CASP1]	9	45%	7	70%	7	88%
H44953	Caspase 4 [CASP4]	4	20%	4	40%	4	50%

Supplementary Table 4. Genes encoding nuclear factors

Gene Bank Access #	Gene Names [Gene Symbol]	Child A (N = 20)		Child B (N = 10)		Child C (N = 8)	
	Up-regulated genes						
AA454656	RNA binding motif protein 4 [RBM4]	5	25%	2	20%	7	88%
AA676804	CCAAT/enhancer binding protein (C/EBP), gamma [CEBPG]	3	15%	1	10%	7	88%
T69468	40S ribosomal protein S4, Y isoform [RPS4Y]	16	80%	8	80%	5	63%
AA130187	Wilms tumor 1 [WT1]	2	10%	1	10%	4	50%
	Down-regulated genes						
AA704459	chromatin assembly factor-I p150 subunit [CHAF1A]	16	80%	9	90%	8	100%
AA455521	E2F transcription factor 5, p130-binding [E2F5]	12	60%	4	40%	8	100%
AA448207	dCMP deaminase (deoxycytidylate deaminase) [DCTD]	9	45%	5	50%	6	75%
AA448261	HMG-I protein isoform; high-mobility group [HMGA1]	6	30%	4	40%	4	50%
AA496438	retinoic acid receptor gamma [RARG]	7	35%	3	30%	4	50%
T66815	H1 histone family member 2 [HIST1H1C]	4	20%	1	10%	4	50%
AA449118	transcription factor A, mitochondrial [TFAM]	7	35%	3	30%	4	50%
AA487235	G/T mismatch binding protein; mutS (E. coli) homolog 6 [MSH6]	8	40%	3	30%	3	38%

Supplementary Table 5. Genes encoding cell contact proteins, extracellular matrix & cytoskeleton

Gene Bank Access #	Gene Names [Gene Symbol]	Child A (N = 20)		Child B (N = 10)		Child C (N = 8)	
	Gene encoding proteins involved in cell contact						
	Up-regulated genes						
AA425217	cadherin 3, type 1, P-cadherin (placental) [CDH3]	15	75%	8	80%	8	100%
AA630328	galectin-3 internal gene [GALIG]	8	40%	6	60%	7	88%
R56219	cadherin 8, type 2 [CDH8]	5	25%	1	10%	5	63%
	Down-regulated genes						
AA035637	junction plakoglobin [JUP]	6	30%	6	60%	8	100%
AA630298	protein tyrosine kinase 2 (focal adhesion kinase) [PTK2]	1	5%	0	0%	6	75%
AA434397	integrin beta-5 subunit [ITGB5]	6	30%	3	30%	5	63%
R89615	protocadherin gamma subfamily C, 3 [PCDHGC3]	3	15%	2	20%	5	63%
AA983530	Vanin 1 [VNN1]; Vascular non-inflammatory molecule 1	6	30%	5	50%	3	38%
	Genes encoding extracellular matrix proteins						
	Up-regulated genes						
H24650	laminin, gamma 1 (formerly LAMB2) [LAMC1]	6	30%	5	50%	7	88%
AA994760	Pro-(alpha)3(V) collagen [COL5A3]	12	60%	5	50%	6	75%
AA452840	fibulin 2 [FBLN2]	8	40%	2	20%	6	75%
AA669222	matrix metalloproteinase 19 [MMP19]	7	35%	1	10%	6	75%
H64138	decorin [DCN]	0	0%	2	20%	5	63%
	Down-regulated genes						
AA857496	matrix metalloproteinase 10 (stromelysin-2) [MMP10]	14	70%	7	70%	7	88%
N22033	collagen, type XI, alpha 2 [COL11A2]	3	15%	3	30%	4	50%
AA464196	hyaluronoglucosaminidase 1 [HYAL1]	1	5%	2	20%	4	50%
	Genes encoding cytoskeleton proteins						
	Up-regulated genes						
AA180013	nebulin-related anchoring protein [NRAP]	7	35%	1	10%	5	63%
	Down-regulated genes						
AA780897	dynamin 2 [DNM2]	3	15%	3	30%	8	100%
R46653	plexin B3 [PLXNB3]	5	25%	1	10%	7	88%
AA411440	villin 2 (ezrin) [VIL2]	4	20%	5	50%	6	75%
AA702663	myosin IXB [MYO9B]	7	35%	4	40%	5	63%

Supplementary Table 6. Genes encoding transporter, calcium-binding proteins and cell membrane-associated proteins

Gene Bank Access #	Gene Names [Gene Symbol]	Child A (N = 20)		Child B (N = 10)		Child C (N = 8)	
Genes encoding transporter molecules							
	Up-regulated genes						
AA708298	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide [ATP5B]	9	45%	6	60%	8	100%
AI359884	T-cell, immune regulator 1, ATPase, H+ transporting, lysosomal V0 protein a isoform 3 [TCIRG1]	4	20%	2	20%	6	75%
	Down-regulated genes						
AA430668	Fc fragment of IgG, receptor, transporter, alpha [FCGRT]	16	80%	5	50%	8	100%
T68568	solute carrier family 10 (sodium/bile acid cotransporter) [SLC10A1]	4	20%	3	30%	8	100%
AA133656	solute carrier family 11 (proton-coupled divalent metal ion transporters) [SLC11A2]	5	25%	1	10%	7	88%
AA702013	solute carrier family 22 (organic cation transporter) [SLC22A1]	1	5%	1	10%	5	63%
Genes encoding calcium-binding proteins							
	Up-regulated genes						
AA598676	reticulocalbin 2, EF-hand calcium binding domain [RCN2]	15	75%	6	60%	8	100%
	Down-regulated genes						
AA778675	calmegin [CLGN]	9	45%	6	60%	5	63%
Genes encoding cell membrane-associated proteins							
	Up-regulated genes						
R62817	stomatin (erythrocyte membrane protein band 7.2) [STOM]	3	15%	2	20%	7	88%
W72596	CAAX box 1 [CXX1]	3	15%	2	20%	7	88%

Supplementary Table 7. Genes encoding proteins associated with immune regulation

Gene Bank Access #	Gene Names [Gene Symbol]	Child A (N = 20)		Child B (N = 10)		Child C (N = 8)	
	Up-regulated genes						
AA464246	HLA class I histocompatibility antigen, CW-4, CW*0401 alpha [HLA-C]	7	35%	3	30%	8	100%
AA054754	interleukin 15 receptor alpha [IL15RA]	3	15%	5	50%	8	100%
AA406028	CD5 antigen (p56-62) [CD5]	5	25%	2	20%	7	88%
AA427664	TIA1 cytotoxic granule-associated RNA binding protein [TIA1]	12	60%	7	70%	5	63%
	Down-regulated genes						
AA256132	interleukin 1 receptor accessory protein [IL1RAP]	12	60%	7	70%	8	100%
T55353	TNF receptor-associated factor 2 [TRAF2]	13	65%	7	70%	6	75%
H70491	C-type lectin-like receptor-2 [CLEC-2]	5	25%	3	30%	6	75%
AA489629	similar to Pre-B cell enhancing factor precursor [PBEF1]	11	55%	2	20%	5	63%
R50354	leukemia inhibitory factor (cholinergic differentiation factor) [LIF]	9	45%	4	40%	5	63%

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