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BASIC RESEARCH

Gene expression profiles of hepatic cell-type specific marker genes in progression of liver fibrosis

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Abstract

AIM: To determine the gene expression profile data for the whole liver during development of dimethylnitrosamine (DMN)-induced hepatic fibrosis.

METHODS: Marker genes were identified for different types of hepatic cells, including hepatic stellate cells (HSCs), Kupffer cells (including other inflammatory cells), and hepatocytes, using independent temporal DNA microarray data obtained from isolated hepatic cells.

RESULTS: The cell-type analysis of gene expression gave several key results and led to formation of three hypotheses: (1) changes in the expression of HSC-specific marker genes during fibrosis were similar to gene expression data in *in vitro* cultured HSCs, suggesting a major role of the self-activating characteristics of HSCs in formation of fibrosis; (2) expression of mast cell-specific marker genes reached a peak during liver fibrosis, suggesting a possible role of mast cells in formation of fibrosis; and (3) abnormal expression of hepatocyte-specific marker genes was found across several metabolic pathways during fibrosis, including sulfur-containing amino acid metabolism, fatty acid metabolism, and drug metabolism, suggesting a mechanistic relationship between these abnormalities and symptoms of liver fibrosis.

CONCLUSION: Analysis of marker genes for specific hepatic cell types can identify the key aspects of fibrogenesis. Sequential activation of inflammatory cells and the self-supporting properties of HSCs play an important role in development of fibrosis.

Key words: Liver fibrosis; Gene expression; Microarray; Dimethylnitrosamine; Marker genes; Hepatic stellate cell; Kupffer cell; Hepatocytes; Metabolic pathway

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INTRODUCTION

The pathological relationship between chronic inflammation and formation of fibrosis has been established in various organs, including the liver, kidney, lung and pancreas. Although liver fibrosis has been studied extensively, the underlying mechanisms remain unclear and drugs to prevent and treat fibrosis are only partially effective. DNA microarray technology offers an approach to this kind of complex problems, and microarray analyses of the whole liver have been reported for liver fibrosis[1,2]. However, these analyses did not address the behavior of individual hepatic cell-types and the interactions of hepatic cells during fibrosis. Therefore, in the current study we identified hepatic cell-specific marker genes that could be used to understand the *in vivo* behavior of each type of hepatic cells during fibrogenesis.

About 70%-80% of hepatic cells are parenchymal hepatocytes, while the non-parenchymal cells are mainly composed of Kupffer cells, hepatic stellate cells (HSC) and sinusoidal endothelial cells (SECs)[3,4]. Kupffer cells are the resident monocytes in liver, and act in phagocytosis of foreign substances such as microorganisms, as well as management of inflammatory processes. Kupffer cells and infiltrated monocytes and lymphocytes are considered to trigger inflammation in the early phase of hepatitis and then maintain chronic inflammation. HSCs control hepatic and cardiovascular contraction, and produce extracellular

matrix (ECM) components and cytokines for repair of organs. HSCs are also believed to have a central role in hepatic fibrosis formation. Hepatocytes fulfill the main functions of the liver, including regulation of nutrition, production of major serum proteins, and elimination of unnecessary materials to maintain homeostasis of the whole body[4].

In the current study, we first examined gene expression profiles of hepatic cells that were isolated at different time points during liver fibrosis. Marker genes specific for different types of hepatic cells were obtained by comparing these profiles and using information from previous studies. DNA microarray data for the whole liver were then interpreted during liver fibrogenesis using the hepatic cell-type specific marker genes. Our results suggest that new pathological properties and intracellular events are associated with each cell type during liver fibrogenesis, and provide candidates for diagnostic markers of liver fibrosis.

MATERIALS AND METHODS

Animals and experimental protocols

Male Sprague-Dawley rats (Charles River Japan, Yokohama, Japan) weighing 160-190 g were housed with unrestricted access to food (CRF-1, Oriental Yeast, Tokyo, Japan) and water in air-conditioned animal quarters with a 12 h light/dark cycle (light between 07:00 and 19:00 h). Hepatic fibrosis was induced by intraperitoneal injection of 0.5% dimethylnitrosamine (DMN; Wako Pure Chemical Industries, Osaka, Japan) at 2 mL/kg of body weight for three consecutive days each week for four weeks. Blood samples were drawn from the inferior vena cava on d 0, 4, 7, 14, 21 and 28, respectively. Liver specimens obtained on these days were dissected and immediately frozen in liquid nitrogen. Fibrosis was confirmed by haematoxylin and eosin (HE) staining of the liver tissue. The hydroxyproline content of liver specimens was determined as previously described by Horie *et al*[5] . Plasma alanine aminotransferase (ATL) and aspartate aminotransferase (AST) were measured using commercial kits (Fuji Film, Tokyo, Japan), and hyaluronic acid levels were determined using a commercial ELISA kit (Fujirebio, Tokyo, Japan). The animal facilities and protocol were reviewed and approved by the Institutional Animal Care and Use Committee of Ajinomoto Co., Inc.

Preparation of HSCs, inflammatory cells (including Kupffer cells) and hepatocytes

HSCs were isolated from rat liver using the pronase-collagenase digestion method as previously reported[6]. Kupffer cell fraction was prepared with an elutriator, using essentially the same method as previously described[6], and hepatocytes were isolated as previously described[7].

Selection of marker genes for hepatic cells

Hepatic cells (HSCs, Kupffer cell fraction and hepatocytes) were isolated from the liver on d 0, 4, 7, 14, 21 and 28 during liver fibrogenesis. The isolated hepatic cells at each time point were subjected to DNA microarray analysis. If the maximum or minimum expression of a gene in hepatocytes was ten times higher or lower than that of the same gene in HSCs and the Kupffer cell fraction, the up- or down-regulated gene was defined as a hepatocyte-specific marker gene. If the maximum or minimum expression of a gene in HSCs was ten times higher or lower than that of the same gene in hepatocytes and three times higher or lower than the same gene in the Kupffer cell fraction, this gene was defined as a HSC-specific marker gene. Similarly, if the maximum or minimum expression of a gene in the Kupffer cell fraction was ten times higher or lower than that of the same gene in hepatocytes and three times higher or lower than the same gene in HSCs, the gene was defined as an inflammatory cell-specific marker gene. An explanation of the use of the term 'inflammatory cell', rather than 'Kupffer cell', was given in the Results section. The ratios used to determine cellular specificity were based on previous reports[8,9]: the number of hepatocytes was about ten times higher than that of the inflammatory cell fraction or HSCs, while the number of Kupffer cells was similar to that of HSCs. Use of a high ratio improved the definition of cellular specificity, but also decreased the number of marker genes. However, we found that these ratios were the most appropriate for identification of a set of genes for analysis of liver fibrogenesis.

Microarray analysis

RNAs both from frozen liver tissues and from each isolated cell type were prepared using Isogen reagent (Nippon Gene), and the quality and quantity of each RNA sample were assessed using an Agilent Bioanalyzer 2100 (Agilent Technologies, Inc.). RNA samples were reverse-transcribed with a poly (dT) oligonucleotide attached to a T7 promoter and copied into dsDNA (Invitrogen). *In vitro* RNA transcription was performed to incorporate biotin-labeled ribonucleotides into the cRNA transcripts using a RNA transcript labeling kit (Enzo Biochem). Some of the RNA (15 g) was utilized for hybridization to a rat genome U34A array (Affymetrix) and a quality assay using test 3 array probe chips was performed according to the manufacturer' s protocol. After hybridization and subsequent washing using the Affymetrix Fluidics Station 400, fluorescence signals amplified with streptavidin phycoerythrin were measured using the Affymetrix scanner, and the results were analyzed using the MicroArray suite software. In the whole liver analysis, two rats and two arrays were used at each time point (d 4, 7, 14, 21 and 28). In the cell-type-specific analysis, one rat which was selected by ALT/AST score and one array were used at each time point (d 4, 7, 14, 21 and 28).

Statistical analysis

Clustering of K-means was performed using the TIGR MeV (MultiExperiment Viewer)[10]. Gene expression profiles in the chronic phase were clustered into 10 patterns using K-means analysis. Clustered genes with a tendency to temporally decrease (clusters 1, 3 and 10) or increase (clusters 7 and 9) were selected as gene markers that had a strong relationship with fibrogenesis.

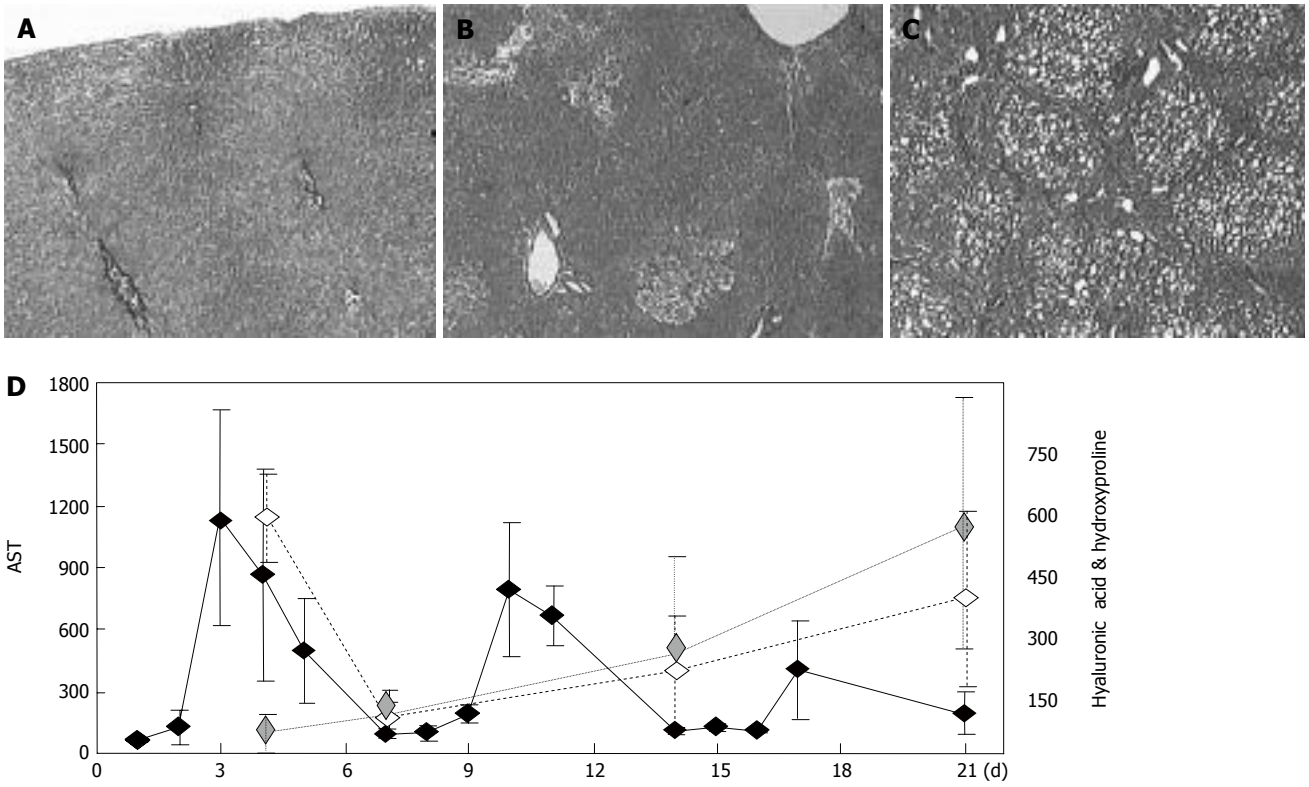

Figure 1 Histological and biochemical analyses of fibrogenesis. **A**-**C**: Histological staining (HE staining) of control liver sections (**A**) and sections obtained on d 4 (**B**) and d 21 (**C**), respectively, after DMN administration; **D**: Biochemical analysis of fibrogenesis showing plasma AST levels (IU/L, solid line), plasma hyaluronic acid levels (ng/mL, dashed line), and liver hydroxyproline levels (ng/mL, dotted line). The x-axis shows the days of fibrogenesis, and each value on the graph is shown as the mean ± SE, $n = 5$.

RESULTS

Time course of gene expression profiles for the whole liver during fibrogenesis

Administration of DMN for three days induced an inflammatory reaction in liver cells simulating the active phase in hepatitis, and the subsequent lack of administration of DMN for four days was used to simulate the remission phase in hepatitis. Repetition of this cycle led to fibrosis in three or four weeks, as shown in Figure 1. AST (GOT) and ALT (GPT) increased on the days of DMN administration and decreased on the days during which DMN was not administered (Figure 1), but both AST and the hyaluronic acid content in serum, a marker of fibrosis[11], gradually increased on days without DMN administration. The inflammatory reaction in periods without DMN administration was weaker than that in periods with DMN-administration, but gradually increased in intensity and response. Based on the behavior shown in Figure 1, gene expression profiles on d 4 (just after a period of DMN administration) were defined as representative of the acute phase response, and those on d 7, 14, 21 and 28 (just before a period of DMN administration) were defined as representative of the chronic phase response.

Marker genes indicating fibrotic activity of HSCs

HSCs were isolated from liver at each time point over the time course of development of DMN-induced fibrosis. Marker genes expressed mainly in HSCs were selected from a DNA microarray analysis, as described in the Materials and Methods, and the selected HSC-specific marker genes are listed in Figure 2. In addition, HSCs isolated from normal rats were cultured *in vitro* for 7 d and DNA microarray analysis of these cells was performed on d 0, 4, and 7, respectively. Marker genes identified in isolated HSCs in the DMN-induced fibrosis model showed the same behavior in the *in vitro* culture, supporting the HSC specificity of the selected marker genes. The behavior of HSC-specific marker genes *in vivo* during fibrogenesis was analyzed using DNA microarray data for the whole liver at each time point during development of DMN-induced fibrosis. These data could indicate the actual behavior of the marker genes *in vivo*, since the data from isolated HSCs might contain some bias due to isolation stimuli. The behavior of HSC-specific marker genes is shown in Figure 2, and supplemental background data are provided in Figure 3. The genes were separated into 2 groups as shown in Figure 2. Group 1 contained marker genes that were linearly up-regulated during fibrogenesis and in the acute inflammation phase, whereas group 2 contained marker genes that were linearly up-regulated during fibrogenesis but not in the acute inflammation phase. Both groups could be further separated into 2 subgroups. Group 1-1 included marker genes that were linearly up-regulated during fibrogenesis and remained in an up-regulated state on d 28, and group 1-2 included marker genes that were also linearly up-regulated during fibrogenesis but then decreased in expression on d 28. Group 2 was similarly separated into groups 2-1 and 2-2. In summary, two groups of HSC-specific marker genes were identified, one in which the genes responded to inflammatory stimuli and the other in which the genes did not respond to such stimuli. Furthermore,

Table 1 Marker genes for hepatic stellate cells (HSCs)

Group	Probe ID	Annotation	Symbol	GenBank	Whole liver						*In vitro* cultured HSC		
					d 0	4	7	14	21	28	d 0	3	7
1	M24067_at	Serine proteinase inhibitor clade E member 1/plasminogen activator inhibitor-1 (PAI-1)	PAI1	M24067	*10.6*	9.3	*1.6*	2.1	*1.8*	**2.1**	*6.0*	29.5	14.8
	M23566exon_s_at	alpha-2-macroglobulin	A2M	M23566	*191.8*	2.3	*0.6*	1.1	*1.0*	**2.3**	*182.9*	2.8	0.8
	M55534mRNA_s_at	Crystallin alpha polypeptide 2/ alpha-crystallin B chain	CRYAB	M55534	13.1	8.9	1.1	1.9	3.2	**3.8**	369.6	3.0	1.5
	Z12298cds_s_at	Decorin	Dcn	Z12298	17.9	2.8	2.5	2.9	3.8	**5.6**	470.1	2.5	2.8
	rc_AA891527_at	Four and a half LIM domains 2	Fhl2	AA891527	*12.7*	3.5	2.7	3.8	5.8	**7.9**	396.3	2.8	2.6
	S57478cds_s_at	Annexin A1/lipocortin I	Anxa1	S57478	14.4	2.6	1.7	1.9	1.8	**2.3**	188.3	3.4	2.5
	D50093_s_at	Prion protein (RaPrP gene for prion protein)	Prnp	D50093	*2.2*	46.0	7.3	19.1	31.4	**37.3**	489.8	2.1	1.8
	rc_AI231472_s_at	Collagen, type 1, alpha 1	Col1a1	AI231472	113.0	1.7	1.0	2.7	3.1	**4.2**	153.1	3.2	5.1
	rc_AA900769_s_at	Vascular alpha-actin/actin, alpha-2, smooth muscle, aorta	ACTA2	AA900769	*24.0*	8.5	1.8	10.1	15.4	**22.2**	56.4	12.1	12.5
	L00382cds_at	Skeletal muscle beta-tropomyosin and fibroblast tropomyosin 1, alternative/Tropomyosin 2	TPM2	L00382	*3.4*	*3.2*	*2.0*	4.1	5.4	**7.4**	1.6	69.0	62.3
	U57362_at	Procollagen, type XII, alpha 1 (collagen XII alpha 1)	Col12a1	U573262	6.3	1.8	2.0	1.9	2.6	**2.8**	47.1	3.1	2.8
	M14656_at	Secreted phosphoprotein 1/ Sialoprotein	Spp1	M14656	7.4	17.8	2.0	2.1	2.4	**20.8**	28.7	34.2	31.9
	rc_AI172064_at	Lectin, galactose binding, soluble 1	Lgals1	AI172064	61.6	2.3	1.2	2.6	**4.3**	3.0	224.3	4.9	3.8
	rc_AA894345_at	Phosphoprotein enriched in astrocytes 15 (predicted)	"Pea15 _predicted"	AA894345	20.5	2.2	1.2	2.0	**2.3**	2.1	112.0	2.1	1.8
	M83107_g_at	Transgelin (SM22-alpha)	Tagln	M83107	*29.3*	3.1	1.6	3.4	**5.1**	**4.6**	*81.7*	14.7	10.0
2	L03294_g_at	Lipoprotein lipase	Lpl	L03294	22.5	1.2	1.6	1.5	2.3	**4.5**	76.9	3.5	7.8
	rc_AI012030_at	Matrix Gla protein	Mgp	AI012030	120.4	1.0	0.9	1.5	2.6	**5.3**	236.2	2.5	4.3
	M80829_at	Troponin T2, cardiac	Tnnt2	M80829	*15.7*	*1.2*	*0.7*	*1.1*	1.8	**2.6**	14.9	18.4	15.5
	M22400_at	Glypican 3 /developmentally regulated intestinal protein (OCI-5)	GPC3	M22400	*3.7*	*0.9*	*2.4*	*3.9*	*7.4*	**10.0**	*8.6*	*0.6*	*1.0*
	D00680_at	Glutathione peroxidase 3/plasma glutathione peroxidase precursor	Gpx3	D00680	*20.5*	1.4	*1.5*	2.7	3.4	**3.9**	104.3	5.1	4.3
	rc_AA800844_s_at	Similar to Loxl protein /LoxL1=lysyl oxidase-like 1	LoxL1	AA800844	*29.3*	1.5	1.5	4.8	7.5	**8.5**	166.0	3.5	5.3
	S77494_s_at	Lysyl oxidase	Lox	S77494	27.5	0.8	0.8	1.9	2.4	**3.0**	26.3	29.1	38.0
	AF030358_g_at	Small inducible cytokine subfamily D, number1/chemokine CX3C motif, ligand 1	CX3CL1	AF030358	*4.6*	*1.0*	2.2	2.3	3.0	**4.0**	24.0	*0.6*	1.5
	X84039_at	Lumican	Lum	X84039	8.1	1.0	2.1	2.1	**2.3**	1.1	52.8	2.3	3.2
	U09540_g_at	Cytochrome P450, family 1, subfamily b, polypeptide 1	Cyp1b1	U09540	*8.1*	*1.0*	*1.3*	1.2	**1.9**	1.6	*29.9*	5.0	6.2

Expression profiles of the whole liver and *in vitro* cultured isolated HSCs were obtained using a rat genome U34A array (Affymetrix). Gene markers for HSCs are listed. For analysis of HSCs in the whole liver, expression intensities are given for d 0, and expression intensity data for d 4, 7, 14, 21 and 28 are shown as ratios to the d 0 expression data. The classification of group1 (2) corresponds to the presence (absence) of up-regulated peak in the acute inflammation phase. For analysis of in vitro cultured HSCs, expression levels for d 0 are also shown, and data for d 4 and 7 are similarly shown as ratios to the expression level on d 0. Italicized values indicate an "absent" call by the Affymetrix software. Bold text indicates the highest ratio in the chronic phase (d 7, 14, 21 and 28). □ between 0.667 and 1.5; ■ ⩾ 1.5; ■ ⩽ 0.667.

HSC-specific marker genes were found that could identify biological changes in HSCs in the late phase of fibrosis.

Marker genes indicating inflammatory activity in immune cell populations

The Kupffer cell fraction was separated from other hepatic cells at each time point during the course of fibrosis development, and marker genes expressed mainly in the Kupffer cell fraction were selected from the DNA microarray analysis. These marker genes are shown in Figure 4, and supplemental background data are provided in Figure 5. Marker genes in the Kupffer cell fraction indicated the presence of other hematopoietic cells, such as mast cells, lymphocytes, erythrocytes and their progenitors in this fraction, as shown in Figures 4 and 5. Since the source cell population for these marker genes could not be confirmed, a particular hematopoietic cell was postulated to be the source of each marker gene based on previous reports as shown in supplemental Table 1. These data indicated that the method used for isolation of Kupffer cells was not appropriate in fibrotic liver, although it was effective for isolation of normal liver cells. However, despite this

Table 2 Marker genes in hepatic stellate cells (HSC)

Group	Probe ID	Annotation	Symbol	Gen Bank	Whole liver						Isolated HSC						*In vitro* cultured HSC		
					d 0	4	7	14	21	28	d 0	4	7	14	21	28	d 0	3	7
Group 1	M24067	Serine proteinase inhibitor clade E member 1/ plasminogen activator inhibitor-1 (PAI-1)	PAI1	M24067	*10.6*	9.3	*1.6*	2.1	*1.8*	**2.1**	565.8	2.6	1.4	2.5	2.2	1.8	*6.0*	29.5	14.8
	M23566	alpha-2-macroglobulin	A2M	M23566	*191.8*	2.3	*0.6*	1.1	*1.0*	**2.3**	*34.3*	*0.5*	*4.1*	7.9	9.4	47.0	*182.9*	2.8	0.8
	M55534	Crystallin alpha polypeptide 2/ alpha-crystallin B chain	CRYAB	M55534	13.1	8.9	1.1	1.9	3.2	**3.8**	619.5	1.9	1.0	1.0	1.5	1.2	369.6	3.0	1.5
	Z12298cds_s_at	Decorin	Dcn	Z12298	17.9	2.8	2.5	2.9	3.8	**5.6**	384.5	2.5	1.6	2.3	2.4	3.5	470.1	2.5	2.8
	rc_AA891527_at	Four and a half LIM domains 2	Fhl2	AA891527	*12.7*	3.5	2.7	3.8	5.8	**7.9**	260.8	2.9	1.5	1.3	2.0	3.7	396.3	2.8	2.6
	S57478cds_s_at	Annexin A1/ lipocortin I	Anxa1	S57478	14.4	2.6	1.7	1.9	1.8	**2.3**	464.2	1.6	0.9	1.3	1.8	2.5	188.3	3.4	2.5
	D50093	Prion protein (RaPrP gene for prion protein)	Prnp	D50093	*2.2*	46.0	7.3	19.1	31.4	**37.3**	361.6	4.3	0.9	1.0	2.9	3.7	489.8	2.1	1.8
	rc_AI231472_s_at	Collagen, type 1, alpha 1	Col1a1	AI231472	113.0	1.7	1.0	2.7	3.1	**4.2**	890.1	0.5	0.8	2.5	2.9	3.8	153.1	3.2	5.1
	rc_AA900769_s_at	Vascular alpha-actin/actin, alpha-2, smooth muscle, aorta	ACTA2	AA900769	*24.0*	8.5	1.8	10.1	15.4	**22.2**	68.0	21.8	18.0	10.8	20.3	15.2	56.4	12.1	12.5
	L00382cds_at	Skeletal muscle beta-tropomyosin and fibroblast tropomyosin 1, alternative/ Tropomyosin 2	TPM2	L00382	*3.4*	*3.2*	*2.0*	4.1	5.4	**7.4**	34.9	2.9	2.3	1.6	6.0	10.2	1.6	69.0	62.3
	U57362_at	Procollagen, type XII, alpha 1 (collagen XII alpha 1)	Col12a1	U57326	6.3	1.8	2.0	1.9	2.6	**2.8**	73.4	2.2	1.1	1.2	2.0	2.9	47.1	3.1	2.8
	M14656_at	Secreted phosphoprotein 1/Sialoprotein	Spp1	M14656	7.4	17.8	2.0	2.1	2.4	**20.8**	8.5	25.1	12.1	11.1	18.6	204.3	28.7	34.2	31.9
	rc_AI172064_at	Lectin, galactose binding, soluble 1	Lgals1	AI172064	61.6	2.3	1.2	2.6	4.3	**3.0**	980.7	2.0	1.3	1.6	1.9	1.6	224.3	4.9	3.8
	AA894345	Similar to MAT1 gene			20.5	2.2	1.2	2.0	2.3	**2.1**	172.0	2.0	0.9	0.9	1.0	0.8	112.0	2.1	1.8
	M83107_g_at	Transgelin (SM22-alpha)	Tagln	M83107	*29.3*	3.1	1.6	3.4	5.1	**4.6**	275.9	8.2	5.3	4.2	7.5	7.2	*81.7*	14.7	10.0
Group 2	L03294_g_at	Lipoprotein lipase	Lpl	L03294	22.5	1.2	1.6	1.5	2.3	**4.5**	130.8	0.5	1.0	1.5	2.2	4.4	76.9	3.5	7.8
	rc_AI012030_at	Matrix Gla protein	Mgp	AI012030	120.4	1.0	0.9	1.5	2.6	**5.3**	1246.7	0.3	0.5	0.6	1.6	2.7	236.2	2.5	4.3
	M80829_at	Troponin T2, cardiac	Tnnt2	M80829	*15.7*	*1.2*	*0.7*	*1.1*	1.8	**2.6**	*17.3*	*0.8*	2.2	3.3	7.7	17.5	14.9	18.4	15.5
	M22400	Glypican 3/ developmentally regulated intestinal protein (OCI-5)	GPC3	M22400	*3.7*	*0.9*	*2.4*	*3.9*	*7.4*	**10.0**	61.9	0.4	0.9	1.5	3.1	9.9	*8.6*	*0.6*	*1.0*
	D00680_at	Glutathione peroxidase 3/ plasma glutathione peroxidase precursor	Gpx3	D00680	*20.5*	1.4	*1.5*	2.7	3.4	**3.9**	134.5	0.7	1.8	3.9	9.2	9.2	104.3	5.1	4.3
	rc_AA800844_s_at	Similar to Loxl protein/ LoxL1=lysyl oxidase-like 1	LoxL1	AA800844	*29.3*	1.5	1.5	4.8	7.5	**8.5**	436.8	0.8	1.4	2.1	3.0	2.5	166.0	3.5	5.3
	S77494_s_at	Lysyl oxidase	Lox	S77494	27.5	0.8	0.8	1.9	2.4	**3.0**	183.1	1.0	3.2	3.4	4.3	3.1	26.3	29.1	38.0

Group	Probe ID	Annotation	Symbol	Gen Bank	Whole liver						Isolated HSC						*In vitro* cultured HSC		
					d 0	4	7	14	21	28	d 0	4	7	14	21	28	d 0	3	7
	AF030358 g at	Small inducible cytokine subfamily D, number1/ chemokine CX3C motif, ligand 1	CX3CL1	AF030358	*4.6*	*1.0*	2.2	2.3	3.0	**4.0**							24.0	*0.6*	1.5
	X84039_at	Lumican	Lum	X84039	8.1	1.0	2.1	2.1	**2.3**	1.1	91.5	0.3	0.8	2.4	1.5	0.3	52.8	2.3	3.2
	U09540_g_at	Cytochrome P450, family 1, subfamily b, polypeptide 1	Cyp1b1	U09540	*8.1*	*1.0*	*1.3*	1.2	**1.9**	1.6	26.5	4.9	0.8	3.9	10.2	14.6	*29.9*	5.0	6.2
Group 3	M31038	Non-RT1 class Ib/ MHC class I non-RT1.A alpha-1-chain		M31038	42.2	1.8	4.0	**6.5**	3.2	3.9	38.3	2.0	10.1	5.0	8.9	0.8	40.7	0.7	0.8
	U44948	Cysteine-and glycine rich-protein 2/smooth muscle cell LIM protein (SmLIM)	CSRP2	U44948	41.9	2.2	1.2	**1.6**	1.1	1.2	406.7	2.6	0.4	0.4	0.5	0.4	202.0	2.9	1.7
	X02601 at	Matrix metalloproteinase 3		X0260	8.6	1.3	**2.0**	1.0	0.9	1.3							597.2	1.1	0.1
Group 4	M15880_at	Neuropeptide Y	Npy	M15880	49.2	4.2	1.0	1.2	1.3	0.8	63.1	2.0	0.7	0.2	1.7	*0.3*	*6.2*	*0.8*	*6.6*
	U50736	Cardiac adriamycin-responsive protein	CARP	U50736	14.3	1.9	1.0	1.0	1.2	1.1	33.7	8.1	2.6	1.6	3.0	4.0	63.6	2.4	1.0

Expression profiles of the whole liver, of isolated HSCs during fibrogenesis, and of in vitro cultured isolated HSCs were obtained using a rat Genome U34A Array (Affymetrix). Marker genes for HSCs are listed. Expression intensities are shown for d 0, and expression intensity data for d 4, 7, 14, 21 and 28 are displayed as ratios to the d 0 expression levels for the analysis of whole liver and isolated HSCs. For in vitro cultured HSCs, the d 0 data are similarly shown as expression intensities, and data on d 4 and 7 are shown as ratios to the expression levels on d 0. Italics indicate an "absent" call by the Affymetrix software, and bold text indicates the highest ratio in the chronic phase (d 7, 14, 21 and 28). □ between 0.667 and 1.5; ■ ⩾ 1.5; ■ ⩽ 0.667.

drawback, the marker genes could be used to study the behavior of inflammatory cells during fibrogenesis, and these genes were therefore defined as inflammatory cell-specific marker genes. The behavior of the inflammatory cell-specific marker genes *in vivo* during fibrogenesis was analyzed using DNA microarray data for the whole liver at each time point during DMN-induced fibrogenesis of marker genes, as summarized in Figures 4 and 5.

The acute phase response was followed by an immunological response, based on the increase in expression of Kupffer cell (or macrophage) markers such as Lyz, Gzmb, and Il1b, as well as surface markers of T cells, such as T-cell receptor, Il2rb, Cd8, Cd76, and Cd45. The up-regulated expression of these genes seemed to indicate activation of Kupffer cells and T lymphocytes, as well as activation of the interaction between these cells, around d 7. Temporary up-regulation of mast cell markers such as chemokines and mast cell proteases indicated the invasion and/or activation of mast cells around d 14, which is of interest since mast cells are known not only to cause acute inflammation, but also to have a role in the induction of chronic inflammation[12-14]. However, whether activation of mast cells is essential for liver fibrosis is unknown. Peak expression of B cell markers such as immunoglobulin occurred on d 21 or d 28, and therefore activation or invasion of B cells seemed to reach its peak in the late phase. Overall, these results showed that inflammatory cell-specific marker genes could be used to monitor the transition of active inflammatory cell populations in fibrosis, and this sequential activation or invasion of inflammatory cells might be related to the stage of fibrotic progression.

Marker genes indicating damage to hepatocytes

Hepatocytes were separated from other hepatic cells at each time point during the course of DMN-induced fibrogenesis, and marker genes expressed mainly in hepatocytes were selected by DNA microarray analysis. The behavior of hepatocyte-specific marker genes *in vivo* during fibrogenesis was analyzed using DNA microarray data for the whole liver at each time point during fibrosis development. Marker genes were categorized based on their functions, as shown in Figure 6. Many abnormally expressed genes were identified and temporal analysis revealed groups of genes showing consistent variation in expression. Gene expression profiles in the chronic phase (d 7, 14, 21 and 28) were clustered into 10 patterns using K-means analysis. Clustered genes with a tendency to temporally decrease (clusters 1, 3 and 10) or increase (clusters 7 and 9) were selected, and then genes were further selected based on a strong correlation coefficient ($\geqslant$ 0.7 or $\leqslant$ -0.7) with fibrosis stage (that is, d 7, 14, 21 and 28). The time courses of expression ratios are shown in Figure 7 as supplemental data. The 41 down-regulated and 19 up-regulated genes that were finally selected are shown in Table 2. These genes appeared to have a strong relationship with progression of fibrosis, and might also share common regulatory expression mechanisms.

Down-regulation of the expression of Cdo1 and Csad showed a strong relationship with fibrotic stage, while expression of Gsta2 was simultaneously up-regulated. Down-regulation of other metabolic enzymes in sulfur-containing metabolic pathways is also shown in Figure 6. These data suggested a broad range of abnormalities in sulfur-containing amino acid metabolic pathways in

Table 3 Marker genes for hematopoietic cells in the Kupffer cell fraction

Group	Probe ID	Annotation	Symbol	GenBank	Whole liver						Cell type	Predicted classification	Reference
					D 0	4	7	14	21	28			
1	M34097_at	Granzyme B/natural killer (NK) cell protease 1 (RNKP-1)	Gzmb	M34097	21.4	1.2	**2.1**	1.1	0.9	0.8	M/L	Kupffer cell, T cell	5
	rc_AA892775_at	Lysozyme	Lyz	AA892775	220.8	9.1	**6.4**	3.4	5.3	**6.4**	M	Kupffer cell	6
	Y12009_at	Chemokine, cc motif, receptor 5	Ccr5	Y12009	11.7	1.8	**2.6**	1.4	1.4	1.2	M/L	T cell	7
	E13732cds_at	Macrophage inflammatory protein-1 alpha receptor/ chemokine, CCmotif, receptor 1/RANTES receptor	Ccr1	E13732	9.4	3.8	**2.3**	1.5	2.2	**2.3**	M/L	T cell	8
	X13044_g_at	CD74 antigen/invariant polpypeptide of major histocompatibility class Ⅱ antigen-associated	Cd74	X13044	131.7	1.9	**13.9**	7.8	7.7	5.6	L	B cell	9, 10
	X04139_s_at	Protein kinase C, beta 1	Prkcb1	X04139	14.5	1.5	**3.7**	2.4	2.2	1.7	M		11
	X03369_s_at	Similar to tubulin, beta	TUBB	X03369	18.6	0.9	**2.0**	1.8	1.1	1.4	M		12
	M98820_at	Interleukin 1 beta	Il1b	M98820	*19.4*	1.6	**3.6**	1.6	1.1	1.1	M	Kupffer cell	13
	U87627_at	Solute carrier family16 (monocarboxylate transporter), member 3	Slc16a3	U87627	24.8	1.9	**1.5**	0.8	1.2	0.9	M/L		14
	D00403_g_at	Interleukin 1 alpha	Il1a	D00403	27.6	0.6	**1.7**	0.8	0.5	0.5	M		15
	rc_AI639534_at	Properdin factor, complement/ Factor P PROPERDIN P FACTOR, COMPLEMENT; PFC	Pfc	AI639534	68.5	1.2	**2.1**	1.6	1.1	0.8	M		16
	X03015_at	CD8 antigen, alpha chain	Cd8a	X03015	3.3	8.5	**15.7**	5.0	9.1	3.8	L	T cell	OMIM
	M18854_at	Similar to T-cell receptor beta-chain/T-cell receptor active beta-chain C-region	---	M18854	*22.7*	*2.0*	**3.7**	2.6	2.6	2.5	L	T cell	
	rc_AA892506_at	Coronin 1A	Coro1a	AA892506	*30.5*	3.8	**4.1**	3.7	3.2	3.6	M/L		OMIM
	M55050_at	Interleukin 2 receptor, beta chain	Il2rb	M55050	48.5	0.9	**1.5**	0.9	0.9	0.8	L	T cell	OMIM
	M30691_at	Ly6-C antigen gene/CD56	Ly6c	M30691	30.2	3.6	**5.0**	1.6	1.7	1.1	L	T cell, NK cell	17
	rc_AA891302_at	Similar to Ser/Thr kinase (BL44)	---	AA891302	*6.9*	*3.2*	***5.4***	4.5	*3.9*	4.1	L	B cell	1
	M10072mRNA_s_at	protein tyrosine phosphatase, receptor type,C/CD45	Ptprc	M10072	8.2	5.9	**4.3**	3.1	3.3	2.7	M		OMIM
	S74141_s_at	Hemopoietic cell kinase/hck tyrosine kinase	Hck	S74141	*42.8*	2.3	**3.5**	2.3	2.5	2.1	M		OMIM
	X52196cds_at	Arachidonate 5-lipoxygenase activating protein	Alox5ap	X52196	34.5	2.3	***2.1***	1.7	1.7	1.8	M	Kupffer cell	2
	U93306_at	Kinase insert domain protein receptor	Kdr	U93306	31.3	0.5	**1.6**	1.2	0.8	0.6	M		18
	U55192_at	Inositol polyphosphate-5-phosphatase D	Inpp5d	U55192	*11.6*	2.1	**3.3**	2.1	2.1	1.6	L/M/Leu		19
	rc_AI178971_at	Similar to alpha globin/ Hemoglobin alpha	(HBA1)	AI178971	141.3	2.4	**0.3**	0.4	0.5	0.5	E		20
	D86297_at	Aminolevulinic acid synthase 2	Alas2	D86297	131.4	2.7	**0.5**	**0.5**	0.8	0.9	E		Entrez gene
	Y07704_g_at	Best5 protein	Best5	Y07704	33.8	2.9	**0.3**	0.5	0.5	0.5	"No information"		
	U50412_at	Phosphatidylinositol 3-kinase, r egulatory subunit, polypeptide 1	Pik3r1	U50412	43.8	1.3	***0.0***	***0.0***	0.5	0.8	M		4
	AB015191_g_at	Rhesus blood group	Rh	AB015191	*22.2*	1.4	***0.3***	***0.3***	*0.5*	0.6	E		Entrez gene
	M94918mRNA_f_at	Hemoglobin beta chain complex/ beta-globin	Hbb	M94918	3283.0	1.4	**0.5**	**0.5**	0.7	0.6	E		OMIM
	J04793_at	Solute carrier family 4 (anion exchanger), member 1	Slc4a1	J04793	58.0	1.2	0.7	**0.6**	1.0	0.7	E		Entrez gene
	U77697_at	Platelet-endothelial cell adhesion molecule/CD31	Pecam	U77697	45.5	0.7	**0.6**	0.9	0.8	0.8	M/L/E		OMIM
2	rc_AI009658_at	Chemokine, CC motif, ligand 5/secreted; RANTES	Ccl5	AI009658	*33.2*	*0.1*	1.8	**3.1**	1.4	*0.3*	L	T cell	OMIM
	rc_AA957923_at	Mast cell protease 2	Mcpt2	AA957923	*10.4*	*1.1*	3.3	**12.3**	8.7	5.8	M	mast cell	26
	U67914_at	Carboxypeptidase A3	Cpa3	U67914	19.3	*0.8*	1.0	**3.5**	2.7	1.6	M	mast cell	27

Group	Probe ID	Annotation	Symbol	GenBank	Whole liver						Cell type	Predicted classification	Reference
					D 0	4	7	14	21	28			
	U67911_s_at	Mast cell protease 9 or mast cell protease 8/mast cell protease 8 precursor (RMCP-8)	"Mcpt9/ Mcpt8"	U67911	*28.9*	*0.6*	2.2	**4.7**	3.0	2.1	M	mast cell	Entrez gene
	U67908_at	Chymase 1, mast cell	Cma1	U67908	*35.2*	*1.0*	*1.2*	**1.8**	1.5	*1.2*	M	mast cell	Entrez gene
	rc_AA957003_at	S100 calcium binding protein A8/ calgranulin A	S100a8	AA957003	6.5	7.8	3.2	**4.2**	1.1	1.4	M/Leu		29, 31
	L18948_at	S100 calcium binding protein A9/ calgranulin B	S100a9	L18948	16.9	5.6	*0.9*	**2.6**	*1.0*	*1.1*	M/Leu		31
	U31598_s_at	Major histocompatibility complex, class Ⅱ, DM alpha (RT1.DMa)	Hla-dma	U31598	98.1	1.9	2.5	**2.6**	2.6	2.2	M/L	Kupffer cell, mast cell	Entrez gene
	U31599_at	Major histocompatibility complex, class Ⅱ, DM beta (RT1. DMb)	Hla-dmb	U31599	20.7	2.3	4.3	3.5	**4.4**	2.8	M/L	Kupffer cell, mast cell	Entrez gene
	L06040_s_at	Arachidonate 12-lipoxygenase	Alox12	L06040	99.8	1.7	*0.2*	***0.1***	*0.3*	0.5	Leu/P		OMIM, 35
3	X06916_at	S100 calcium-binding protein A4 Mts1	S100a4	X06916	18.2	7.1	2.7	1.8	2.2	**3.1**	M/L		36
	M28671_at	Similar to Ig gamma-2B chain C region (rearranged IgG-2b)	---	M28671	*14.7*	*0.6*	*0.8*	*1.0*	**3.3**	*0.7*	L	B cell	
	rc_AI234828_g_at	Immunoglobulin heavy chain, alpha polypeptide	Igha	AI234828	28.8	0.6	1.0	1.7	2.0	**2.4**	L	B cell	
	X53517_at	CD37 antigen	Cd37	X53517	*32.5*	2.7	1.9	2.1	**2.3**	**2.3**	L	B cell	OMIM
	X58294_at	Carbonic anhydrase 2	Ca2	X58294	63.8	2.0	0.8	1.0	1.6	**2.1**	M/L/E		37, 38
	AF072411_g_at	cd36 antigen	Cd36	AF072411	67.0	1.3	1.2	0.9	1.2	**1.8**	M/E		OMIM
	U75689_s_at	Deoxyribonuclease I-like 3/ DNase gamma	Dnase1l3	U75689	106.6	0.1	1.1	1.1	0.5	**0.2**	M		40
	X73371_at	Fc receptor, IgG, low affinity IIb/ Fc gamma receptor	Fcgr2b	X73371	190.7	0.5	0.8	1.1	0.7	**0.5**	M/L/Leu	B cell, mast cell	OMIM
	L04672_s_at	Adrenomedullin receptor	Admr	L04672	147.0	0.5	0.8	0.9	0.7	**0.4**	L	T cell possibly	17, 4
4	AF041083_at	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	Slc11a1	AF041083	*15.1*	2.7	*0.7*	*0.9*	*1.0*	*0.9*	M		Entrez gene
	D14015_g_at	Cyclin E	CCNE1	D14015	27.1	1.5	1.3	0.9	0.9	1.0	M		44
	U31367_at	Myelin and lymphocyte protein /myelin protein MVP17	Mal	U31367	*19.9*	2.4	*1.0*	1.1	1.2	*0.7*	L	T cell	43

Expression profiles of the whole liver and the isolated Kupffer cell fraction during fibrogenesis were obtained using a rat genome U34A array (Affymetrix). Marker genes for hematopoietic cells in the Kupffer cell fraction are listed. Expression intensities are given for d 0, and expression intensity data for d 4, 7, 14, 21 and 28 are shown as ratios to the d 0 expression data for analysis of hematopoietic cells in whole liver. The classification of genes into groups 1-4 and corresponds to the maximum change of expression at the different time points on d 7, 14, 21, 28 and 4, respectively. Italicized values indicate an "absent" call by the Affymetrix software. The bold values indicate the highest or lowest ratio in the chronic phase (d 7, 14, 21 and 28). □ between 0.667 and 1.5; ■ ⩾ 1.5; ■ ⩽ 0.667. Cell types in the inflammatory cell fraction are as follows: M: Monocytes and their progenitors; L: Lymphocytes and their progenitors; E: Erythrocytes and their progenitors; P: Platelets and their progenitors; Leu: Other kinds of leukocytes and their progenitors.

fibrosis. Regarding other genes, Hao2 and Hpcl2 had a role in fatty acid oxidation in peroxisome, while Amacr was associated with beta-oxidation of pristanoyl-CoA and C27-bile acyl-CoAs. The down-regulation of these genes and other metabolic enzymes related to fatty acid oxidation (Figure 6) suggested abnormalities in the fatty acid oxidation process in fibrosis. The expression of Amacr might be related to that of the nuclear receptor subfamily 0, group B, member 2 (Nr0b2), and changes in NrOb2 expression may affect one of the key molecules in cholesterol biosynthesis. Up-regulation of Gk and down-regulation of Pepck1, PC and Slc37a4 suggested abnormalities in gluconeogenesis, glycogen storage and glycolysis, while down-regulation of Ste and Hsd17b2 suggested an abnormality of estrogen metabolism in the liver. Decreased expression of many Cyp drug metabolism enzymes was also found in progression of fibrosis, and abnormalities in hormonal signaling were suggested by the down-regulation of Inhbe, Ghr and Dio1. These results showed that identification of hepatocyte-specific marker genes could allow analysis of functional changes in fibrosis, and all the identified abnormalities might have major effects on hepatic function.

DISCUSSION

Marker genes for HSCs

Markers of HSCs such as Acta 2, Cryab, Spp1, Prnp, and Pai-1 were strongly up-regulated on d 4, and then quickly decreased in expression following a gradual up-regulation. On the other hand, other HSC markers such as Gpc3, Lox, and Mgp did not show marked up-regulation on d 4, but their expression level increased linearly during fibrogenesis. Therefore, HSCs may be associated with events in two

Table 4 Marker genes in hematopoietic cells in the Kupffer cell fraction

Group	Probe ID	Annotation	Symbol	GenBank	Whole liver						Isolated Kupffer cells						Cell type	Predicted classification	Ref.
					D 0	4	7	14	21	28	d 0	4	7	14	21	28			
1	M34097_at	Granzyme B/natural killer (NK) cell protease 1 (RNKP-1)	Gzmb	M34097	21.4	1.2	**2.1**	1.1	0.9	0.8	42.1	1.9	7.5	7.6	3.2	1.3	M/L	Kupffer cell, T1 cell	
	rc_AA892775_at	Lysozyme	Lyz	AA892775	220.8	9.1	**6.4**	3.4	5.3	**6.4**	1164.3	5.5	2.9	4.1	2.9	3.9	M	Kupffer cell	2.3
	Y12009_at	Chemokine, cc motif, receptor 5	Ccr5	Y12009	11.7	1.8	**2.6**	1.4	1.4	1.2	29.4	11.9	8.7	6.8	4.5	5.4	M/L	T cell	4
	E13732cds_at	Macrophage inflammatory protein-1 alpha receptor/chemokine, CCmotif, receptor 1/RANTES receptor	Ccr1	E13732	9.4	3.8	**2.3**	1.5	2.2	**2.3**	40.6	11.8	1.7	2.3	1.8	4.0	M/L	T cell	5
	X13044_g_at	CD74 antigen/ invariant polpypeptide of major histocompatibility class II antigen-associated	Cd74	X13044	131.7	1.9	**13.9**	7.8	7.7	5.6	1672.0	1.9	2.4	3.1	2.3	2.6	L	B cell	2,6,7
	X04139_s_at	Protein kinase C, beta 1	Prkcb1	X04139	14.5	1.5	**3.7**	2.4	2.2	1.7	33.5	2.2	3.7	4.5	2.5	4.9	M		8
	X03369_s_at	Similar to tubulin, beta	TUBB	X03369	18.6	0.9	**2.0**	1.8	1.1	1.4	28.6	0.9	2.5	10.5	4.2	5.3	M		9
	M98820_at	Interleukin 1 beta	Il1b	M98820	*19.4*	1.6	**3.6**	1.6	1.1	1.1	884.8	6.8	2.7	2.6	2.2	2.8	M	Kupffer cell	10,11,13
	U87627_at	Solute carrier family16 (monocarboxylate transporter), member 3	Slc16a3	U87627	24.8	1.9	**1.5**	0.8	1.2	0.9	52.0	10.4	2.8	9.3	5.4	3.2	M/L		14,15,16
	D00403_g_at	Interleukin 1 alpha	Il1a	D00403	27.6	0.6	**1.7**	0.8	0.5	0.5	1395.6	1.9	1.1	0.4	0.4	0.6	M		17,18
	rc_AI639534_at	Properdin factor, complement/Factor P PROPERDIN P FACTOR, COMPLEMENT; PFC	Pfc	AI639534	68.5	1.2	**2.1**	1.6	1.1	0.8	49.8	11.9	5.1	5.8	8.7	7.3	M		19
	X03015_at	CD8 antigen, alpha chain	Cd8a	X03015	3.3	8.5	**15.7**	5.0	9.1	3.8	31.3	2.3	4.4	4.5	2.2	0.9	L	T cell	OMIM
	M18854_at	Similar to T-cell receptor beta-chain/ T-cell receptor active beta-chain C-region	---	M18854	*22.7*	*2.0*	**3.7**	2.6	2.6	2.5	37.9	1.0	7.1	15.5	9.3	4.3	L	T cell	
	rc_AA892506_at	Coronin 1A	Coro1a	AA892506	*30.5*	3.8	**4.1**	3.7	3.2	3.6	216.3	1.4	3.5	5.2	3.8	3.0	M/L		OMIM
	M55050_at	Interleukin 2 receptor, beta chain	Il2rb	M55050	48.5	0.9	**1.5**	0.9	0.9	0.8	64.7	2.0	4.1	4.1	2.9	1.7	L	T cell	OMIM
	M30691_at	Ly6-C antigen gene /CD56	Ly6c	M30691	30.2	3.6	**5.0**	1.6	1.7	1.1	173.8	3.7	2.1	2.6	1.8	1.4	L	T cell, NK cell	20,22
	rc_AA891302_at	Similar to Ser/Thr kinase (BL44)	---	AA891302	*6.9*	3.2	***5.4***	4.5	*3.9*	4.1	57.3	1.1	2.6	6.9	4.6	5.8	L	B cell	23
	M10072mRNA_s_at	Protein tyrosine phosphatase, receptor type,C/CD45	Ptprc	M10072	8.2	5.9	**4.3**	3.1	3.3	2.7	70.6	6.9	4.6	4.7	1.6	4.3	M		24
	S74141_s_at	Hemopoietic cell kinase hck tyrosine kinase	Hck	S74141	*42.8*	2.3	**3.5**	2.3	2.5	2.1	114.0	10.6	4.1	4.5	4.2	4.7	M		OMIM
	X52196cds_at	Arachidonate 5-lipoxygenase activating protein	Alox5ap	X52196	*34.5*	2.3	***2.1***	1.7	1.7	1.8	82.4	9.1	3.2	8.4	5.3	6.5	M	Kupffer cell	25,26
	U93306_at	kinase insert domain protein receptor	Kdr	U93306	31.3	0.5	**1.6**	1.2	0.8	0.6	1516.6	0.1	0.2	0.1	0.1	0.1	M		27,28,29
	U55192_at	Inositol polyphosphate-5-phosphatase D	Inpp5d	U55192	*11.6*	2.1	**3.3**	2.1	2.1	1.6	56.8	3.3	2.6	4.4	3.4	3.4	L/M/Leu		OMIM
	rc_AI178971_at	Similar to alpha globin/Hemoglobin alpha	(HBA1)	AI178971	141.3	2.4	**0.3**	0.4	0.5	0.5	18.2	*0.8*	*0.6*	*0.5*	15.6	*1.2*	E		OMIM
	D86297_at	Aminolevulinic acid synthase 2	Alas2	D86297	131.4	2.7	**0.5**	**0.5**	0.8	0.9	29.9	*0.2*	4.1	5.2	40.9	4.9	E		Entrez gene
	Y07704_g_at	Best5 protein	Best5	Y07704	33.8	2.9	**0.3**	0.5	0.5	0.5	668.1	*0.0*	0.2	0.1	0.4	0.2	No information		
	U50412_at	Phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1	Pik3r1	U50412	43.8	1.3	***0.0***	***0.0***	0.5	0.8	*5.0*	14.0	*2.6*	*0.5*	*2.1*	3.4	M		30,31,32
	AB015191_g_at	Rhesus blood group	Rh	AB015191	*22.2*	1.4	***0.3***	***0.3***	*0.5*	0.6	*14.1*	*0.5*	4.6	4.8	40.7	4.8	E		Entrez gene

Group	Probe ID	Annotation	Symbol	GenBank	Whole liver						Isolated Kupffer cells						Cell type	Predicted classification	Ref.
					D 0	4	7	14	21	28	d 0	4	7	14	21	28			
	M94918mRNA_f_at	Hemoglobin beta chain complex/ beta-globin	Hbb	M94918	3283.0	1.4	**0.5**	**0.5**	0.7	0.6	1212.2	0.3	2.0	2.7	4.8	2.9	E		OMIM
	J04793_at	Solute carrier family 4(anion exchanger), member 1	Slc4a1	J04793	58.0	1.2	0.7	**0.6**	1.0	0.7	29.0	1.4	3.7	3.1	18.6	2.8	E		Entrez gene
	U77697_at	Platelet-endothelial cell adhesion molecule/CD31	Pecam	U77697	45.5	0.7	**0.6**	0.9	0.8	0.8	821.2	0.3	0.3	0.2	0.3	0.3	M/L /E		OMIM
2	rc_AI009658_at	Chemokine, CC motif, ligand 5/secreted; RANTES	Ccl5	AI009658	*33.2*	*0.1*	1.8	**3.1**	1.4	*0.3*	456.2	0.5	0.4	1.6	1.1	0.3	L	T cell	OMIM
	rc_AA957923_at	Mast cell protease 2	Mcpt2	AA957923	*10.4*	*1.1*	3.3	**12.3**	8.7	5.8	23.8	2.9	3.8	89.6	45.0	43.7	M	mast cell	33,34
	U67914_at	Carboxypeptidase A3	Cpa3	U67914	19.3	*0.8*	1.0	**3.5**	2.7	1.6	*24.6*	1.4	2.0	31.4	13.7	19.6	M	mast cell	35,36,37
	U67911_s_at	Mast cell protease 9 or mast cell protease 8/ mast cell protease 8 precursor (RMCP-8)	"Mcpt9/ Mcpt8"	U67911	*28.9*	*0.6*	2.2	**4.7**	3.0	2.1	81.2	1.2	2.9	24.9	13.4	8.4	M	mast cell	Entrez gene
	U67908_at	Chymase 1, mast cell	Cma1	U67908	*35.2*	*1.0*	*1.2*	**1.8**	1.5	*1.2*	*32.4*	*1.2*	*1.4*	16.1	11.3	6.7	M	mast cell	Entrez gene
	rc_AA957003_at	S100 calcium binding protein A8/ calgranulin A	S100a8	AA957003	6.5	7.8	3.2	**4.2**	1.1	1.4	31.6	6.2	2.6	8.9	7.4	1.0	M/ Leu		38,39
	L18948_at	S100 calcium binding protein A9/ calgranulin B	S100a9	L18948	16.9	5.6	*0.9*	**2.6**	*1.0*	*1.1*	48.9	6.7	4.4	8.9	10.7	1.3	M/ Leu		40
	U31598_s_at	Major histocompatibility complex, class II, DM alpha (RT1.DMa)	Hla-dma	U31598	98.1	1.9	2.5	**2.6**	**2.6**	2.2	172.1	4.5	3.3	4.9	4.3	6.6	M/L	Kupffer cell, mast cell	Entrez gene
	U31599_at	Major histocompatibility complex, class II, DM beta (RT1.DMb)	Hla-dmb	U31599	20.7	2.3	4.3	3.5	**4.4**	2.8	33.5	8.3	8.7	8.8	14.4	15.5	M/L	Kupffer cell, mast cell	Entrez gene
	L06040_s_at	Arachidonate 12-lipoxygenase	Alox12	L06040	99.8	1.7	*0.2*	***0.1***	*0.3*	0.5	*9.2*	*3.3*	*3.0*	7.6	35.1	*2.5*	Leu/ P		41,42,43
3	X06916_at	S100 calcium-binding protein A4 Mts1	S100a4	X06916	18.2	7.1	2.7	1.8	2.2	**3.1**	132.0	9.5	1.5	4.0	2.8	2.3	M/L		45
	M28671_at	Similar to Ig gamma-2B chain C region (rearranged IgG-2b)	---	M28671	*14.7*	*0.6*	*0.8*	*1.0*	**3.3**	*0.7*	61.7	3.3	5.8	13.9	3.9	3.8	L	B cell	
	rc_AI234828_g_at	Immunoglobulin heavy chain, alpha polypeptide	Igha	AI234828	28.8	0.6	1.0	1.7	2.0	**2.4**	905.3	0.5	0.9	0.9	0.4	0.7	L	B cell	
	X53517_at	CD37 antigen	Cd37	X53517	*32.5*	2.7	1.9	2.1	**2.3**	**2.3**	105.9	3.2	1.8	3.6	2.7	2.1	L	B cell	OMIM
	X58294_at	Carbonic anhydrase 2	Ca2	X58294	63.8	2.0	0.8	1.0	1.6	**2.1**	*36.7*	0.4	7.2	5.8	35.8	6.3	M/ L/E		46,47
	AF072411_g_at	CD36 antigen	Cd36	AF072411	67.0	1.3	1.2	0.9	1.2	**1.8**	77.9	9.4	4.0	2.8	3.5	4.6	M/E		OMIM
	U75689_s_at	Deoxyribonuclease I-like 3/DNase gamma	Dnase1l3	U75689	106.6	0.1	1.1	1.1	0.5	**0.2**	2583.7	0.1	0.2	0.2	0.2	0.1	M		48
	X73371_at	Fc receptor, IgG, low affinity IIb/Fc gamma receptor	Fcgr2b	X73371	190.7	0.5	0.8	1.1	0.7	**0.5**	2913.7	0.5	0.4	0.4	0.4	0.2	M/ L/ Leu	B cell, mast cell	OMIM
	L04672_s_at	Adrenomedullin receptor	Admr	L04672	147.0	0.5	0.8	0.9	0.7	**0.4**	2004.8	0.2	0.3	0.2	0.4	0.1	L	T cell possibly	49,50
4	AF041083_at	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	Slc11a1	AF041083	*15.1*	2.7	*0.7*	*0.9*	*1.0*	*0.9*	77.2	1.8	1.6	4.6	2.0	2.1	M		Entrez gene
	D14015_g_at	Cyclin E	CCNE1	D14015	27.1	1.5	1.3	0.9	0.9	1.0	37.6	1.4	2.0	1.5	7.6	1.3	M		
	U31367_at	Myelin and lymphocyte protein/ myelin protein MVP17	Mal	U31367	*19.9*	2.4	*1.0*	1.1	1.2	*0.7*	32.2	14.2	2.4	3.9	2.2	1.8	L	T cell	51

Expression profiles of the whole liver and the isolated Kupffer cell fraction during fibrogenesis were obtained using a rat Genome U34A Array (Affymetrix). Marker genes of hematopoietic cells in the Kupffer cell fraction are listed. Expression intensities are shown for d 0, and the expression intensity data for d 4, 7, 14, 21 and 28 are displayed as ratios to the d 0 expression levels. Italics indicate an "absent" call by the Affymetrix software. Bold values indicate the highest or lowest ratio in the chronic phase (d 7, 14, 21 and 28). □ between 0.667 and 1.5; ■ ⩾ 1.5; ■ ⩽ 0.667. The cell types in the inflammatory cell fraction are as follows: M: Monocytes and their progenitors; L: Lymphocytes and their progenitors; E: Erythrocytes and their progenitors; P: Platelets and their progenitors; Leu: Other kinds of leukocytes and their progenitors.

Table 5 References used in the definition of types of hematopoietic cells in the Kupffer cell fraction

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The reference numbers correspond to the numbers in Table 4 in the supplementary data.

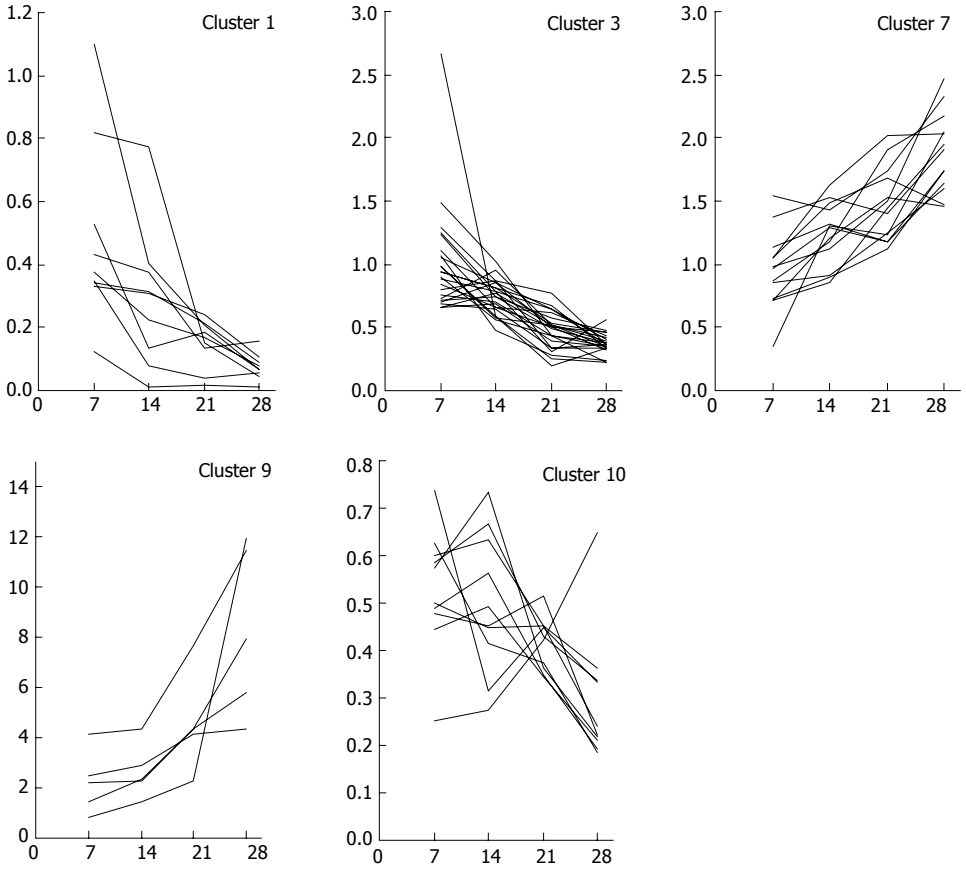

Figure 2 Cluster of genes that increased or decreased in expression with progression of fibrogenesis. Gene expression profiles in the chronic phase (d 7, 14, 21 and 28) were clustered into 10 patterns using K-means analysis. Clustered genes with a tendency to temporally decrease (clusters 1, 3 and 10) or increase (clusters 7 and 9) were selected as gene markers that had a strong relationship with fibrogenesis.

Table 6 Marker genes for hepatocytes

Probe ID	Functional category	Annotation	Symbol	GenBank	UniGene	Whole Liver						Isolated Hepatocytes					
						d 0	4	7	14	21	28	d 0	4	7	14	21	28
U68168_at	Amino acid metabolism	Kynureninase (L-kynurenine hydrolase)	Kynu	U68168	Rn.10575	487.4	0.4	1.0	1.2	0.9	1.2	981.6	0.5	0.8	0.5	0.5	0.4
Z50144_g_at		Aminoadipate aminotransferase	Aadat	Z50144	Rn.11133	336.1	0.4	0.7	0.9	0.9	1.0	360.3	0.6	0.9	0.7	0.6	0.8
AF056031_at		Kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	Kmo	AF056031	Rn.35029	292.1	0.4	0.9	1.0	0.9	1.1	483.1	0.6	0.5	0.7	0.5	0.5
D44494_at		3-hydroxyan-thranilate 3,4-dioxygenase	Haao	D44494	Rn.48675	1071.6	0.5	0.7	0.9	0.7	0.8	948.6	0.6	1.0	0.8	0.8	0.8
M84648mRNA_s_at		Dopa decarboxylase	Ddc	M84648		219.5	0.4	0.5	0.8	0.6	0.6	241.3	0.4	1.1	0.6	0.7	0.3
J02827_at		Branched chain ketoacid dehydrogenase E1, alpha polypeptide	Bckdha	J02827	Rn.3489	194.2	0.5	0.4	0.7	0.7	0.7	164.1	0.7	0.9	0.6	1.0	1.3
rc_AI168942_at		Branched chain keto acid dehydrogenase E1, beta polypeptide	Bckdhb	AI168942	Rn.15623	335.2	0.3	0.6	0.7	0.7	0.7	248.2	0.4	1.0	1.2	1.6	1.9
rc_AI102838_s_at		Isovaleryl coenzyme A dehydrogenase	Ivd	AI102838	Rn.147	345.5	0.4	0.6	0.5	0.7	0.8	439.6	0.6	0.7	0.6	0.7	0.7
M93401_at		Aldehyde dehydrogenase family 6, subfamily A1	Aldh6a1	M93401	Rn.2098	564.1	0.7	0.6	1.2	1.0	1.2	796.3	0.5	0.5	0.8	0.6	0.8
J05499_at		Glutaminase 2 (liver, mitochondrial)	Gls2	J05499	Rn.10202	254.0	0.6	0.9	0.9	0.8	1.3	410.8	0.6	0.4	0.6	0.4	2.1
D10354_s_at		Glutamic pyruvic transaminase 1, soluble	Gpt1	D10354	Rn.6318	229.0	0.6	0.9	0.9	1.2	1.6	201.7	0.9	0.9	1.2	1.0	4.1
J04171_at		Glutamate oxaloacetate transaminase 1	Got1	J04171	Rn.5819	262.9	1.0	0.7	0.9	1.1	1.7	342.5	0.7	0.8	0.8	0.6	4.9
M58308_at		Histidine ammonia lyase	Hal	M58308	Rn.10037	244.9	1.1	1.5	1.4	1.2	2.3	413.3	1.3	0.7	0.6	0.9	2.7
X13119cds_s_at		Serine dehydratase	Sds	X13119		425.9	0.7	0.7	1.1	0.7	1.2	116.7	0.6	0.2	1.2	0.3	23.0
rc_AA892112_g_at		Proline dehydrogenase (oxidase) 2 (predicted)	(Prodh2)	AA892112	Rn.4247	423.3	0.5	0.7	1.0	0.9	0.9	597.2	0.5	0.9	0.7	1.1	1.0
rc_AA892345_at		Dimethylglycine dehydrogenase precursor	Dmgdh	AA892345	Rn.3646	219.5	0.4	0.7	0.8	1.0	0.8	276.6	0.5	0.8	0.7	0.8	0.8
AF067650_at		Sarcosine dehydrogenase	Sardh	AF067650	Rn.37484	133.1	0.7	0.5	0.8	0.6	0.6	112.7	0.5	0.5	0.7	0.6	1.3
J03588_at		Guanidinoacetate methyltransferase	Gamt	J03588	Rn.33890	2017.0	0.3	0.7	0.8	0.6	0.7	1684.6	0.4	1.0	0.8	1.0	0.7
X06150cds_at		Glycine N-methyltransferase	Gnmt	X06150	Rn.11142	605.4	0.2	0.7	0.6	0.5	0.6	801.9	0.3	0.5	0.5	0.3	0.8
X15734_at		Methionine adenosyl-transferase I, alpha	Mat1a	X15734	Rn.10418	1290.3	0.5	0.5	0.6	0.6	0.5	438.8	0.6	0.6	0.6	0.8	2.9
AF038870_at		Betaine-homocysteine methyltransferase	Bhmt	AF038870	Rn.11406	2239.0	0.3	0.7	0.8	0.8	0.8	3346.2	0.3	0.7	0.8	0.7	0.8
M59861_at		Formyltetra-hydrofolate dehydrogenase	Fthfd	M59861	Rn.2328	554.2	0.5	0.9	1.3	1.4	1.0	924.3	0.5	1.0	0.9	1.0	1.0
rc_AA942685_at		Cytosolic cysteine dioxygenase 1	Cdo1	AA942685	Rn.2589	1815.9	0.4	0.9	0.8	0.5	0.5	1960.7	0.4	0.6	0.6	0.6	0.5
M64755_at		Cysteine sulfinic acid decarboxylase	Csad	M64755	Rn.43232	394.3	0.1	0.4	0.5	0.3	0.2	403.3	0.2	0.7	0.3	0.6	0.1
rc_AI012802_at		Hydroxyacyl glutathione hydrolase	Hagh	AI012802	Rn.11048	537.6	0.6	0.6	0.9	0.7	0.9	657.2	0.5	0.8	0.7	0.7	0.8

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						d 0	4	7	14	21	28	d 0	4	7	14	21	28
E01415cds_s_at		Glutathione S-transferase, mu type 3	Gstm3	E01415		314.8	0.2	0.7	0.5	0.5	0.6	456.3	0.2	0.4	0.5	0.4	0.5
rc_AI235747_at		Glutathione transferase YA subunit	Gsta5	AI235747	Rn.10460	77.2	0.6	2.1	1.5	1.2	0.8	94.0	0.7	0.8	1.3	0.8	0.3
rc_AA945082_at		Glutathione-S-transferase, alpha type2	Gsta2	AA945082	Rn.40574	10.3	2.0	2.2	2.3	4.4	7.9	24.1	1.4	2.5	3.1	2.2	2.6
K03041mRNA_s_at		Ornithine transcarbamylase	Otc	K03041		219.7	0.4	0.7	1.0	0.8	0.9	259.2	0.4	0.5	0.8	0.4	0.5
X12459_at		Arginosuccinate synthetase	Ass	X12459	Rn.5078	2507.3	0.6	0.7	0.7	0.7	0.8	2828.7	0.3	0.6	0.6	0.5	1.4
J03959_g_at		Urate oxidase	Uox	J03959	Rn.11330	74.9	0.4	0.4	0.7	0.7	0.6	67.0	0.4	0.9	0.7	1.0	1.0
M33648_at	Cholesterol synthesis & bile acid synthesis	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	Hmgcs2	M33648	Rn.29594	3672.7	0.4	0.5	0.7	0.5	0.5	3097.5	0.6	0.8	0.6	0.9	0.8
rc_AI180442_at		Farensyl diphosphate synthase	Fdps	AI180442	Rn.2622	193.8	0.2	0.6	0.6	0.5	0.5	212.0	0.4	1.2	0.8	1.6	0.2
M95591_at		Farnesyl diphosphate farnesyl transferase 1	Fdft1	M95591	Rn.3252	594.7	0.2	0.3	0.6	0.4	0.5	480.9	0.4	0.8	0.7	0.9	0.2
AB016800_at		7-dehydro-cholesterol reductase	Dhcr7	AB016800	Rn.228	198.3	0.3	0.7	1.1	0.8	1.1	312.6	0.6	0.9	0.8	1.0	0.4
D86745exon_s_at		Nuclear receptor subfamily 0, group B, member 2	Nr0b2	D86745		201.3	0.2	0.6	0.4	0.4	0.2	147.5	0.4	0.3	0.4	0.6	0.5
D14989_f_at		Similar to Alcohol sulfotransferase (Hydroxysteroid sulfotransferase) (ST) (ST-60)	LOC361510	D14989	Rn.40365	334.8	0.3	1.0	0.9	0.4	1.4	372.1	1.0	0.7	1.5	0.8	0.5
D43964_at		Bile acid-Coenzyme A: amino acid N-acyltransferase	Baat	D43964	Rn.11129	743.8	0.5	1.5	1.2	0.9	0.7	1038.5	0.8	0.8	0.6	0.8	0.6
E12625cds_at		Sterol-C4-methyl oxidase-like	Sc4mol	E12625		579.8	0.1	0.2	0.5	0.2	0.3	452.9	0.1	0.4	0.4	0.8	0.1
L16995_at		Sterol regulatory element binding factor 1	Srebf1	L16995		116.7	0.7	1.0	1.5	1.2	1.6	227.6	0.2	0.6	1.3	0.7	0.3
rc_AA866264_s_at	Steroid hormone synthesis & metabolism	Similar to 20-alpha -hydroxysteroid dehydrogenase	---	AA866264	Rn.14713	37.8	0.5	1.0	1.1	1.5	2.5	100.8	0.2	0.7	0.8	0.6	1.2
S76489_s_at		Sulfotransferase, estrogen preferring estrogen sulfotransferase	"Ste, ste2"	S76489		1787.7	0.3	0.7	0.9	0.5	0.3	1919.8	0.3	0.8	0.4	0.3	0.1
J05035_at		Steroid 5 alpha-reductase 1	Srd5a1	J05035	Rn.4620	483.3	0.4	1.1	1.0	0.7	1.1	1175.7	0.6	0.3	0.6	0.8	0.2
U72497_at		Fatty acid amide hydrolase	Faah	U72497	Rn.10619	493.0	0.5	0.7	0.9	0.7	0.6	439.6	0.5	1.0	0.7	1.1	0.7
D17310_s_at		3-alpha-hydroxysteroid dehydrogenase	LOC191574	D17310	Rn.10021	440.6	0.5	0.8	0.8	0.6	0.8	576.3	0.8	0.8	0.7	0.7	0.5
rc_AI105448_at		Hydroxysteroid 11-beta dehydrogenase 1	Hsd11b1	AI105448	Rn.888	2057.5	0.5	0.8	0.7	0.7	0.6	2047.0	0.3	0.9	0.6	0.8	0.7
X91234_at		Hydroxysteroid (17-beta) dehydrogenase 2	Hsd17b2	X91234	Rn.10515	1060.3	0.2	0.8	0.8	0.2	0.0	158.0	0.4	0.8	0.3	1.7	0.5
rc_AI101743_s_at		Hydroxysteroid (17-beta) dehydrogenase 4	Hsd17b4	AI101743	Rn.2082	151.5	0.9	0.5	1.1	0.7	0.9	267.3	0.5	0.4	0.8	0.5	1.1
U89280_at		Hydroxysteroid (17-beta) dehydrogenase 9	Hsd17b9	U89280	Rn.10857	901.9	0.6	0.7	0.9	1.4	1.9	734.2	1.4	0.7	2.0	1.6	3.3
rc_AA893495_at		Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 6	Serpina6	AA893495	Rn.2374	1880.6	0.2	0.9	0.8	0.6	0.6	2046.0	0.4	0.7	0.6	0.9	0.3

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S80431_s_at		Aldo-keto reductase family 1, member D1	Akr1d1	S80431		284.2	0.7	0.5	1.4	0.8	1.0	786.7	0.3	0.2	0.8	0.3	0.9
rc_AI172293_at		Sterol-C4-methyl oxidase-like	Sc4mol	AI172293	Rn.7167	677.2	0.2	0.3	0.6	0.3	0.4	694.9	0.2	0.6	0.4	0.9	0.1
U89905_at	Lipid biosynthesis, fatty acid metabolism & lipid transport	Alpha-methylacyl-CoA racemase	Amacr	U89905	Rn.2590	590.0	0.1	0.6	0.7	0.4	0.2	303.5	0.9	0.9	0.5	0.9	0.6
J02749_at		Acetyl-Coenzyme A acyltransferase 1 (peroxisomal 3-oxoacyl-Coenzyme A thiolase)	Acaa1	J02749	Rn.8913	130.3	0.4	0.8	1.4	0.9	1.7	79.4	2.1	2.3	1.9	2.4	3.8
X95189_at		Acyl-Coenzyme A oxidase 2, branched chain	Acox2	X95189	Rn.10622	510.4	0.2	0.7	1.2	0.5	0.3	797.7	0.2	0.3	0.5	0.6	0.4
X95188_at		Acyl-Coenzyme A oxidase 3, pristanoyl	Acox3	X95188	Rn.10546	73.8	0.5	*0.4*	0.7	*0.6*	*0.4*	56.7	0.4	0.4	0.7	0.9	1.0
K03249_at		Enoyl-Coenzyme A, hydratase/ 3-hydroxyacyl Coenzyme A dehydrogenase	Ehhadh	K03249	Rn.3671	198.2	0.2	0.9	1.0	0.8	0.8	372.1	0.4	0.7	0.9	0.9	0.3
U64451_at		Acyl-Coenzyme A dehydrogenase, short/branched chain	Acadsb	U64451	Rn.44423	91.6	0.6	0.6	0.7	0.5	0.6	82.9	0.5	0.1	0.6	0.2	0.9
AF044574_g_at		2-4-dienoyl-Coenzyme A reductase 2, peroxisomal	Decr2	AF044574	Rn.7879	212.3	0.5	1.0	1.4	0.9	1.5	399.5	0.9	1.0	0.7	0.8	0.6
rc_AA893239_at		2-hydroxy-phytanoyl -Coenzyme A lyase	Hpcl2	AA893239	Rn.21425	480.5	0.1	0.4	0.4	0.1	0.2	236.1	0.5	0.3	0.3	0.4	0.6
rc_AI175764_s_at		Stearoyl-Coenzyme A desaturase 1	Scd1	AI175764	Rn.1023	52.7	0.2	0.7	1.5	1.7	0.8	47.2	0.1	2.6	5.3	2.3	0.1
rc_AA893242_g_at		Acyl-CoA synthetase long-chain family member 1	Acsl1	AA893242	Rn.6215	415.1	0.3	0.5	1.0	0.7	0.8	822.4	0.2	0.3	0.6	0.4	0.4
rc_AI171506_at		Malic enzyme 1	Me1	AI171506	Rn.3519	39.6	0.6	1.1	1.4	0.9	1.8	52.7	0.8	1.7	1.1	1.8	0.3
X70223_at		Peroxisomal membrane protein 2	Pxmp2	X70223	Rn.10292	1059.1	0.3	0.8	0.7	0.6	0.6	907.9	0.7	0.9	0.8	1.0	0.5
rc_AI232087_at		Hydroxyacid oxidase 2 (long chain)	Hao2	AI232087	Rn.10417	731.5	0.4	1.2	0.7	0.6	0.5	693.5	0.2	0.9	0.4	0.3	0.4
U10697_s_at		Carboxylesterase 1	Ces1	U10697	Rn.82692	574.2	0.4	1.2	0.8	0.7	0.6	691.0	0.9	0.9	0.6	1.1	0.4
M20629_s_at		Esterase 2	Es2	M20629	Rn.2549	2513.3	0.2	0.6	0.6	0.5	0.2	2429.2	0.5	0.7	0.6	1.0	0.0
AB010635_s_at		Carboxylesterase 2 (intestine, liver)	Ces2	AB010635	Rn.14535	42.1	9.7	4.2	4.4	7.7	11.5	45.3	15.2	4.9	7.0	10.1	15.6
L46791_at		Carboxylesterase 3	Ces3	L46791	Rn.34885	223.0	0.1	1.0	0.6	0.5	0.4	463.6	0.1	0.8	0.4	0.6	0.0
M16235_at		Lipase, hepatic	Lipc	M16235	Rn.1195	760.7	0.4	0.7	0.9	0.7	0.6	826.2	0.6	0.7	0.9	1.1	0.5
X03468_at		Apolipoprotein A-Ⅱ	Apoa2	X03468	Rn.10309	2543.4	0.3	0.8	0.6	0.8	0.7	3332.3	0.5	0.6	0.4	0.7	0.2
M00002_at		Apolipoprotein A-Ⅳ	Apoa4	M00002	Rn.15739	298.8	0.4	0.6	0.6	0.5	0.6	411.7	0.6	0.7	0.2	0.8	0.5
U53873cds_at		Apolipoprotein B	Apob	U53873		731.2	0.8	0.2	0.4	0.3	0.2	246.2	0.1	0.1	0.7	0.0	1.2
rc_AA945171_at		Apolipoprotein C-Ⅳ	Apoc4	AA945171	Rn.33157	720.3	0.7	0.6	0.9	0.7	0.8	1066.0	0.6	0.8	0.5	0.5	0.7
rc_AA893213_at		Apolipoprotein M	Apom	AA893213	Rn.262	1525.1	0.6	0.7	0.8	0.7	0.8	1912.6	0.8	0.9	0.6	0.8	0.6
U02096_at		Fatty acid binding Protein 7, brain	Fabp7	U02096	Rn.10014	190.4	0.3	1.1	0.5	0.3	0.2	129.4	0.7	0.8	0.3	0.3	0.1
U26033_at		Carnitine O-octanoyl-transferase	Crot	U26033	Rn.4896	100.7	0.9	1.7	2.3	1.9	2.0	142.9	1.9	2.2	2.8	1.1	1.9
K03045cds_r_at	Retiniod synthesis & metabolism	Retinol binding protein 4, plasma	Rbp4	K03045		3156.1	0.6	0.6	0.6	0.6	0.4	2863.7	0.5	0.6	0.6	0.9	0.6
U33500_g_at		Retinol dehydrogenase type Ⅱ (RODHⅡ)	RoDHII	U33500	Rn.37873	255.1	0.7	1.3	0.9	0.8	0.4	95.0	0.8	2.1	0.4	0.7	3.2
U18762_at		Retinol dehydrogenase type Ⅲ	Rdh3	U18762	Rn.31786	72.9	0.2	0.3	0.3	0.4	0.6	110.2	0.5	0.3	0.8	0.9	0.3

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						d 0	4	7	14	21	28	d 0	4	7	14	21	28
X53588_at	Glucolysis & Gluconeogenesis	Glucokinase	Gck	X53588	Rn.10447	56.7	0.5	0.4	1.3	1.2	2.0	218.3	0.1	0.3	0.3	0.1	0.1
M86235_at		Ketohexokinase	Khk	M86235		813.7	0.4	0.8	1.0	0.8	0.9	701.4	0.5	1.4	1.0	1.3	0.9
rc_AA945442_at		Glucokinase regulatory protein	Gckr	AA945442	Rn.7863	248.0	0.3	0.8	1.2	1.0	1.0	449.8	0.4	1.0	0.6	0.8	0.4
rc_AA892395_s_at		Aldolase B	Aldob	AA892395	Rn.10592	2821.0	0.5	0.8	0.9	0.7	0.8	3179.1	0.6	0.8	0.7	0.7	0.9
AB002558_at		Glycerol-3 -phosphate dehydrogenase 1 (soluble)	Gpd1	AB002558	Rn.44452	164.6	0.9	0.7	1.1	0.7	0.7	102.5	0.6	0.9	1.5	0.9	1.0
X05684_at		Pyruvate kinase, liver and RBC	Pklr	X05684		111.0	0.2	0.8	0.8	0.8	0.4	107.4	0.3	1.1	0.7	0.9	0.2
U32314_at		Pyruvate carboxylase	Pc	U32314	Rn.11094	398.3	0.3	0.7	0.8	0.5	0.4	456.4	0.5	0.8	0.5	0.8	0.5
X15580complete_seq_s_at		6-phosphofructo-2-kinase/ fructose-2,6-biphosphatase 1	Pfkfb1	X15580	Rn.10115	262.8	0.1	0.3	0.4	0.5	0.4	89.2	0.4	1.0	0.5	0.8	0.7
rc_AA892799_i_at		Glyoxylate reductase/ hydroxypyruvate reductase (predicted)	(Grhpr)	AA892799	Rn.7815	669.4	0.4	0.4	1.0	0.7	0.7	561.6	0.4	0.8	0.7	0.8	0.5
L37333_s_at		Glucose-6-phosphatase, catalytic	G6pc	L37333	Rn.10992	864.9	0.2	0.8	1.2	0.3	0.4	1915.9	0.4	0.5	0.6	1.0	0.6
X04069_at		Liver glycogen phosphorylase	Pygl	X04069	Rn.21399	129.7	0.5	1.0	1.4	1.1	0.8	248.9	0.5	0.8	0.7	0.7	0.3
K03243mRNA_s_at		Phosphoenol-pyruvate carboxykinase 1	Pck1	K03243		2955.1	0.5	0.7	0.6	0.3	0.3	2764.6	0.9	0.3	0.5	0.9	1.0
AF080468_at		Solute carrier family 37 (glycerol-6-phosphate transporter), member 4	Slc37a4	AF080468	Rn.1592	681.2	0.2	0.7	0.7	0.5	0.4	609.2	0.4	0.7	0.6	1.0	0.4
rc_AI030175_s_at		Sorbitol dehydrogenase	Sord	AI030175	Rn.11334	762.4	0.7	0.7	1.1	0.9	0.8	1108.8	0.5	0.6	0.8	0.7	0.6
D63704_g_at	Nucleotide-related enzymes	Dihydropy-rimidinase	Dpys	D63704	Rn.10586	388.7	0.4	0.7	0.7	0.5	0.5	466.5	0.6	0.7	0.7	0.8	0.3
M97662_at		Ureidopropionase, beta	Upb1	M97662	Rn.11110	1487.6	0.4	0.5	0.7	0.9	0.7	1151.1	0.4	1.0	0.8	1.1	0.8
D85035_at		Dihydropy-rimidine dehydrogenase	Dpyd	D85035	Rn.17564	230.4	0.3	0.8	0.7	0.8	1.0	238.1	0.5	0.9	0.9	0.5	1.7
AF041066_at		Ribonuclease, RNase A family 4	Rnase4	AF041066	Rn.22804	1617.5	0.3	1.0	0.9	0.6	0.4	1395.3	0.5	0.8	0.8	1.1	0.6
M57507_at		Guanylate cyclase 1, soluble, beta 2	Gucy1b2	M57507	Rn.10933	85.8	0.3	0.5	0.8	0.5	0.6	95.5	0.3	0.9	0.4	0.5	0.3
E01184cds_s_at	Drug-metabolism	Cytochrome P450, family 1, subfamily a, polypeptide 2	Cyp1a2	E01184		1518.5	0.1	0.3	0.3	0.2	0.1	1898.4	0.4	0.4	0.3	0.4	0.0
J04187_at		Cytochrome P450, subfamily 2A, polypeptide 1	Cyp2a2	J04187	Rn.9867	798.2	0.5	0.8	0.8	0.7	0.6	1022.0	0.4	0.8	0.6	0.6	0.5
K01721mRNA_s_at		Cytochrome P450, family 2, subfamily b, polypeptide 15	Cyp2b15	K01721	Rn.2287	462.6	0.2	0.7	0.4	0.3	0.7	1031.8	0.4	0.3	0.5	0.2	0.1
X79081mRNA_f_at		Cytochrome P450, subfamily IIC (mephenytoin 4-hydroxylase)	Cyp2c	X79081		591.2	0.0	0.4	0.2	0.2	0.1	411.3	0.1	0.2	0.1	0.1	0.1
J02861mRNA_s_at		Cytochrome P450 2c13	Cyp2c13	J02861	Rn.32070	1659.9	0.5	1.6	1.0	0.8	0.6	1914.7	0.5	0.9	0.7	0.6	0.7
AB008424_s_at		Cytochrome P450, family 2, subfamily d, polypeptide 13	Cyp2d13	AB008424	Rn.32106	1316.3	0.4	0.9	1.0	0.7	0.7	1998.3	0.5	0.8	0.5	0.8	0.5
AB008423_s_at		Cytochrome P450, family 2, subfamily d, polypeptide 26	Cyp2d26	AB008423	Rn.40137	3830.8	0.7	1.0	0.7	0.7	0.6	4520.5	0.7	0.7	0.6	0.8	0.7
S48325_s_at		Cytochrome P450, family 2, subfamily e, polypeptide 1	Cyp2e1	S48325		5249.5	0.4	0.6	0.5	0.5	0.5	6219.9	0.4	0.5	0.5	0.6	0.6

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AF017393_at		Cytochrome P450, family 2, subfamily f, polypeptide 2	Cyp2f2	AF017393	Rn.10817	180.5	0.5	0.7	1.0	0.9	1.0	234.2	0.8	0.9	0.7	1.0	0.5
U40004_s_at		Cytochrome P450, family 2, subfamily j, polypeptide 9	Cyp2j9	U40004	Rn.37480	336.9	0.3	0.7	0.5	0.5	0.4	440.5	0.5	0.7	0.6	0.9	0.3
X62086mRNA_s_at		"Cytochrome P450, subfamily 3A, polypeptide 3 cytochrome P450, family 3, subfamily a, polypeptide 11 cytochrome P-450PCN (PNCN inducible)"	Cyp3a3, Cyp3a11, RGD:628626	X62086		3742.3	0.3	0.5	0.5	0.4	0.5	3344.0	0.5	0.5	0.6	0.4	0.6
M13646_s_at		Cytochrome P450, family 3, subfamily a, polypeptide 11	Cyp3a11	M13646	Rn.37424	2198.3	0.4	1.1	0.7	0.5	0.5	2356.8	0.8	0.7	0.6	0.5	0.1
U46118_at		Cytochrome P450, family 3, subfamily a, polypeptide 13	Cyp3a13	U46118	Rn.10489	37.7	*0.7*	1.5	1.4	1.7	2.3	28.7	2.8	2.2	1.7	2.2	7.3
D38381_s_at		Cytochrome P450, 3a18	RGD:628709	D38381	Rn.32085	444.8	0.6	1.9	1.0	0.9	1.3	682.8	0.8	0.9	0.7	0.4	1.1
U39206_at		Cytochrome P450 4F4	RGD:708363	U39206	Rn.10170	357.7	0.3	0.8	0.7	0.5	0.7	311.9	0.4	0.6	0.8	0.7	0.9
M94548_at		Cytochrome P450, family 4, subfamily F, polypeptide 2	Cyp4f2	M94548	Rn.5722	1637.4	0.6	1.0	0.9	0.8	0.8	2192.8	0.7	0.7	0.6	0.9	0.4
J05460_s_at		Cytochrome P450, family 7, subfamily a, polypeptide 1	Cyp7a1	J05460	Rn.10737	305.0	0.1	1.3	1.5	0.4	0.6	224.3	*0.1*	0.6	1.3	0.5	*0.1*
M21208mRNA_s_at		Cytochrome P450, family 17, subfamily a, polypeptide 1	Cyp17a1	M21208	Rn.10172	36.6	0.3	2.7	0.6	0.3	*0.2*	67.4	0.9	0.5	0.4	0.5	1.7
U17697_s_at		Cytochrome P450, subfamily 51	Cyp51	U17697	Rn.6150	355.0	0.2	0.5	0.7	0.5	0.5	660.8	0.4	0.9	0.6	1.2	0.1
M13506_at		Liver UDP-glucuronosy-ltransferase, phenobarbital-inducible form	Udpgtr2	M13506	Rn.9969	198.2	1.7	1.4	0.7	0.9	0.8	424.1	2.7	0.5	0.8	1.1	0.6
D38069exon_s_at		UDP glycosyl-transferase 1 family, polypeptide A6	Ugt1a6	D38069		63.8	0.6	0.5	1.4	0.9	0.8	83.9	0.6	0.6	1.7	1.0	0.5
rc_AA818122_f_at		Sulfotransferase family, cytosolic, 2A, dehydroepian-drosterone (DHEA)-preferring, member 1	Sth2	AA818122	Rn.2151?	1199.3	0.3	1.3	0.8	0.3	0.6	1674.5	0.9	0.3	0.7	0.5	0.3
L22339_g_at		Sulfotransferase family 1A, member 2	Sult1a2	L22339	Rn.9937	1303.6	0.4	1.1	0.9	0.7	0.8	1587.5	0.4	0.9	0.6	0.4	0.3
rc_AA926193_at		Sulfotransferase family, cytosolic, 1C, member 2	RGD:621064	AA926193	Rn.22471	119.3	0.2	0.7	0.5	0.6	0.5	99.8	0.2	1.0	0.5	0.4	0.2
X56228_g_at		Thiosulfate sulfurtransferase	Tst	X56228	Rn.6360	1476.9	0.4	0.5	0.8	0.7	0.7	1565.7	0.4	0.8	0.6	0.8	0.5
rc_AA892821_at		Aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase)	RGD:620311	AA892821	Rn.8548	184.3	0.4	0.7	1.0	0.8	0.8	223.4	0.6	1.0	0.6	0.6	0.8
AF045464_s_at		Aldo-keto reductase family 7, member A3 (aflatoxin aldehyde reductase)	Akr7a3	AF045464	Rn.6043	337.0	1.5	2.0	1.6	1.4	1.7	240.8	2.9	2.1	1.6	2.0	2.6

Probe ID	Functional category	Annotation	Symbol	GenBank	UniGene	Whole Liver						Isolated Hepatocytes					
						d 0	4	7	14	21	28	d 0	4	7	14	21	28
X65083cds_at		Epoxide hydrolase 2, cytoplasmic	Ephx2	X65083	Rn.54495	44.5	0.8	0.8	1.6	0.6	0.2	22.3	2.6	2.5	1.3	0.2	4.3
AF001898_at		Aldehyde dehydrogenase family 1, member A1	Aldh1a1	AF001898	Rn.6132	304.5	4.1	2.5	2.9	4.1	4.4	752.5	2.2	1.4	1.2	1.5	2.9
M23995_g_at		Aldehyde dehydrogenase family 1, subfamily A4	Aldh1a4	M23995	Rn.74044	167.4	2.1	0.8	0.1	1.2	3.1	148.8	2.1	1.3	5.0	1.6	2.1
rc_AI172017_at		Aldehyde dehydrogenase 2	Aldh2	AI172017	Rn.2300	1125.0	0.5	0.7	0.7	0.6	0.6	1227.2	0.6	1.0	0.6	1.1	0.5
X90710_at		Alcohol dehydrogenase 4 (class II), pi polypeptide	Adh4	X90710	Rn.10302	201.0	0.6	1.5	1.0	0.4	0.4	182.8	0.9	0.5	0.5	0.6	0.5
rc_AA817846_at		3-hydroxy-butyrate dehydrogenase (heart, mitochondrial)	Bdh	AA817846	Rn.36635	574.7	0.3	0.9	0.8	0.7	0.3	723.4	0.3	0.4	0.2	0.7	0.3
rc_AA892382_at		Camello-like 1	Cml1	AA892382	Rn.3643	59.8	0.3	0.7	0.7	0.4	0.3	44.2	0.5	0.7	0.5	0.6	0.4
M26125_at		Epoxide hydrolase 1	Ephx1	M26125	Rn.3603	1661.4	1.7	1.8	1.6	1.5	1.7	2083.7	2.3	1.6	1.8	2.2	1.7
M84719_at		Flavin containing monooxygenase 1	Fmo1	M84719	Rn.867	320.7	0.0	0.5	0.1	0.2	0.1	466.1	0.0	0.5	0.1	0.1	*0.0*
rc_AA817964_s_at		Paraoxonase 1	Pon1	AA817964	Rn.20732	4497.8	0.4	0.8	0.7	0.7	0.6	4715.3	0.5	0.7	0.7	0.8	0.5
M31363mRNA_f_at		Sulfotransferase family, cytosolic, 2A, dehydroepian-drosterone (DHEA) -preferring, member 1	Sth2	M31363	Rn.2151	2071.8	0.3	1.0	0.6	0.2	0.3	2228.8	0.7	0.3	0.4	0.4	0.3
M11670_at	Anti-oxidant enzymes	Catalase	Cat	M11670	Rn.3001	697.4	0.4	1.4	0.9	0.7	0.7	723.0	0.6	0.8	0.9	0.8	0.5
U94856_at		Paraoxonase 1	Pon1	U94856	Rn.20732	3127.6	0.4	0.7	0.7	0.6	0.6	3370.6	0.5	0.7	0.7	0.8	0.5
M15481_at	Growth factors & their receptors	Insulin-like Growth factor 1	Igf1	M15481	Rn.6282	534.3	0.4	0.3	0.6	0.4	0.4	367.7	0.6	0.7	0.6	0.7	0.6
M58634_at		Insulin-like Growth factor binding protein 1	Igfbp1	M58634	Rn.34026	521.1	1.8	0.7	0.3	0.4	0.3	914.9	1.8	0.3	0.3	0.9	0.9
J04486_at		Insulin-like growth factor binding protein 2	Igfbp2	J04486	Rn.6813	17.4	2.4	1.5	2.3	4.3	5.8	13.1	6.0	3.3	4.9	5.2	14.6
rc_AA924289_s_at		Insulin-like growth factor binding protein, acid labile subunit	Igfals	AA924289	Rn.7327	217.0	0.3	0.6	0.8	0.5	0.6	234.0	0.3	0.5	0.8	0.8	0.4
AF089825_at		Inhibin beta E	Inhbe	AF089825	Rn.30020	156.2	0.3	0.8	0.9	0.3	0.4	147.0	0.6	1.2	0.8	1.1	0.2
S49003_s_at		Growth hormone receptor	Ghr	S49003		1081.0	0.2	0.5	0.4	0.5	0.4	1080.8	0.2	0.7	0.7	0.9	0.4
AF076619_at		Growth factor receptor bound protein 14	Grb14	AF076619	Rn.30028	210.0	0.7	0.6	0.7	0.4	0.6	221.9	0.8	0.8	0.8	0.5	0.6
M32167_g_at		Vascular endothelial growth factor A	Vegfa	M32167	Rn.1923	42.1	1.0	0.6	0.7	0.4	0.5	36.3	0.8	0.4	0.8	0.7	0.7
M37394_at		Epidermal growth factor receptor	Egfr	M37394	Rn.37227	134.6	0.4	0.6	0.7	0.5	0.4	116.6	0.7	0.8	0.6	0.9	0.5
L48060_s_at		Prolactin receptor	Prlr	L48060	Rn.9757	64.0	0.3	0.6	0.8	1.0	1.5	133.3	0.5	0.5	0.8	1.3	1.1
rc_AA892251_at		Arginine vasopressin receptor 1A	Avpr1a	AA892251	Rn.32282	204.4	0.1	0.4	1.0	1.0	0.6	455.0	0.3	0.7	0.6	1.1	*0.1*
L32132_at		Lipopoly-saccharide binding protein	Lbp	L32132	Rn.48863	54.1	3.1	0.9	1.2	1.9	2.2	39.4	8.1	1.5	1.9	3.1	5.7
L13025UTR#1_f_at		Polymeric immunoglobulin receptor	Pigr	L13025		421.3	0.5	0.5	0.5	0.5	0.7	1974.1	0.6	0.6	0.8	0.8	0.7
D14869_s_at		Prostaglandin E receptor 3 (subtype EP3)	Ptger3	D14869	Rn.10361	72.3	0.7	0.5	0.5	0.3	0.4	46.6	0.4	0.4	0.5	0.5	0.5
K01934mRNA#2_at		Thyroid hormone responsive protein	Thrsp	K01934		1556.7	0.2	0.8	1.0	0.8	0.7	1201.3	0.1	1.1	1.0	1.4	0.1
X57999cds_at		Deiodinase, iodothyronine, type I	Dio1	X57999	Rn.42914	139.8	0.4	0.6	0.7	0.4	*0.3*	168.8	0.4	0.5	0.2	0.6	*0.2*

Probe ID	Functional category	Annotation	Symbol	GenBank	UniGene	Whole Liver						Isolated Hepatocytes					
						d 0	4	7	14	21	28	d 0	4	7	14	21	28
X76456cds_at	Hepatic secretory proteins	Afamin	Afm	X76456		3150.5	0.3	0.8	0.8	0.7	0.5	3565.8	0.5	0.8	0.9	0.9	0.3
X02361_at		Alpha-fetoprotein	Afp	X02361	Rn.9174	*46.5*	*0.4*	*0.7*	1.2	1.5	1.5	35.4	0.5	1.8	1.2	1.3	0.6
rc_AA817854_s_at		Ceruloplasmin	Cp	AA817854	Rn.32777	418.5	0.9	0.5	1.1	0.8	0.8	362.8	1.1	0.9	1.6	0.5	1.1
X86178mRNA_g_at		Alpha-2-glycoprotein 1, zinc	Azgp1	X86178		871.9	0.5	0.7	0.7	0.7	0.8	1252.1	0.5	0.6	0.9	0.8	0.9
M27434_s_at		Alpha-2u globulin PGCL1	LOC259246	M27434		6719.1	0.2	1.1	0.4	0.2	0.1	4046.4	0.6	0.8	0.1	0.5	0.0
J00738_s_at		Alpha-2u globulin PGCL4	RGD:708508	J00738	Rn.81155	527.0	0.0	0.1	0.0	0.0	*0.0*	53.4	0.1	0.2	0.1	0.1	0.2
X51615_at		Pregnancy-zone protein	Pzp	X51615		55.0	0.7	0.9	0.8	0.6	0.8	63.3	0.6	0.6	2.0	0.9	0.4
rc_AA945608_at		Serum amyloid P-component	Sap	AA945608	Rn.1902	1278.3	0.6	0.8	0.7	0.7	0.7	1286.9	0.9	0.9	0.6	0.9	0.6
rc_AI102562_at		Metallothionein	Mt1a	AI102562	Rn.54397	8150.5	0.6	0.5	0.5	0.5	0.2	2209.3	2.2	0.6	0.6	1.2	1.2
X86561cds#2_at	Blood-function	Fibrinogen, alpha Polypeptide	Fga	X86561		1512.9	0.8	0.5	0.6	0.6	0.5	1223.6	1.8	1.1	1.0	0.9	0.5
D21215cds_s_at		Coagulation factor X	F10	D21215		641.3	0.4	0.6	0.9	0.7	0.8	850.3	0.6	0.7	0.9	1.0	0.6
U20194_g_at		Complement component 8, beta polypeptide	C8b	U20194	Rn.10152	1279.6	0.5	0.5	0.6	0.5	0.4	954.2	0.7	0.7	0.5	1.0	0.6
M62832_at		Plasminogen	Plg	M62832		1783.6	0.5	0.5	0.7	0.7	0.6	1618.8	0.5	0.8	0.9	1.2	0.5
M12112mRNA#3_s_at		Angiotensinogen	Agt	M12112		780.9	0.5	0.6	0.9	0.8	0.7	1279.3	0.6	1.0	0.7	1.1	0.9
L00117_at	Protease & protease inhibitors	Elastase 1, pancreatic	Ela1	L00117		132.6	0.0	0.3	0.1	0.0	0.1	74.5	*0.2*	0.3	*0.1*	*0.0*	*0.0*
rc_AI230712_at		Subtilisin-like endoprotease	Pace4	AI230712	Rn.950	63.8	1.0	0.5	0.9	1.2	1.1	78.2	1.2	0.9	0.5	1.0	1.0
AF097723_s_at		Plasma glutamate carboxypeptidase	Pgcp	AF097723	Rn.17112	519.4	0.3	0.6	0.6	0.4	0.4	464.5	0.4	0.8	0.6	0.7	0.4
X70900_at		Hepsin	Hpn	X70900	Rn.11139	799.9	0.5	0.7	0.8	0.7	0.7	792.6	0.6	0.8	0.7	1.0	0.7
rc_AA946503_at		Lipocalin 2	Lcn2	AA946503	Rn.11303	26.4	4.0	*0.8*	1.4	2.3	12.0	7.1	126.5	5.2	35.9	4.3	157.1
X69834_at		Serine (or cysteine) proteinase inhibitor, clade A, member 3M	Serpina3m	X69834	Rn.10424	875.6	0.5	0.8	0.8	1.0	1.0	752.4	1.2	0.5	1.6	2.6	0.5
D00752_at		Serine protease inhibitor	Spin2a	D00752	Rn.34396	5647.3	0.3	0.8	0.7	0.6	0.4	6388.0	0.4	0.7	0.5	0.8	0.1
M35299_s_at		Serine protease inhibitor, Kazal type 1	Spink1	M35299	Rn.9767	55.7	0.6	0.8	0.7	0.4	0.5	39.6	0.5	0.3	0.7	0.1	1.1
X16273cds_at		Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	Serpina1	X16273		6888.3	0.6	1.0	0.7	0.6	0.6	6788.4	1.0	0.8	0.6	0.7	0.9
rc_AA893552_at		Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4	Serpina4	AA893552	Rn.11152	813.0	0.4	0.7	0.6	0.7	0.6	809.8	0.5	1.0	0.6	1.0	0.4
M63991_at		Serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antipeptidase, antitrypsin), member 7	Serpina7	M63991	Rn.9948	25.6	1.8	2.2	1.5	3.8	1.6	10.7	7.1	7.9	4.6	1.8	1.6
M22993cds_s_at		"Murinoglobulin 1 homolog (mouse) alpha-1-inhibitor III similar to Murinoglobulin 1 homolog murinoglobulin 2"	"Mug1, LOC297568, LOC297572, RGD:1302962"	M22993		979.0	0.5	0.3	0.8	0.5	0.5	1427.7	0.1	0.1	0.6	0.2	0.6
V01216_at	Cell surface proteins & structural proteins	Orosomucoid 1	Orm1	V01216	Rn.10295	1695.5	1.5	1.3	1.0	1.2	1.3	1097.2	3.1	1.9	2.3	2.2	4.6
X05023_at		Mannose-binding protein C (liver)	Mbl2	X05023	Rn.9667	107.8	0.5	0.8	1.0	0.8	0.7	179.0	0.6	0.9	0.8	0.9	0.7
AF080507_at		Mannose-binding protein mRNA	---	AF080507		462.7	0.6	1.2	1.3	1.2	1.1	739.8	0.7	1.0	1.2	1.1	0.6
K02817cds_s_at		Asialoglycoprotein receptor 1	Asgr1	K02817		544.5	0.8	0.6	0.7	0.6	0.6	661.3	1.0	0.8	0.6	0.9	0.7

Probe ID	Functional category	Annotation	Symbol	GenBank	UniGene	Whole Liver						Isolated Hepatocytes					
						d 0	4	7	14	21	28	d 0	4	7	14	21	28
U82612cds_g_at		Fibronectin 1	Fn1	U82612		626.7	1.0	0.4	0.8	0.6	0.5	607.1	0.4	0.4	0.8	0.8	0.6
M81687_at		Syndecan 2	Sdc2	M81687	Rn.11127	438.7	0.4	0.7	0.9	0.7	0.6	542.6	0.6	0.7	0.8	0.7	0.3
AF090134_at		Lin-7 homolog a (C. elegans)	Lin7a	AF090134	Rn.31766	86.2	0.8	0.6	0.9	0.5	0.7	69.9	1.2	1.4	1.0	0.8	0.5
X04070_at		Gap junction membrane channel protein beta 1	Gjb1	X04070	Rn.10444	1214.5	0.5	0.5	0.6	0.6	0.5	1377.4	0.4	0.7	0.6	1.0	0.6
rc_AA799879_at		Synaptogyrin 1	Syngr1	AA799879	Rn.11188	75.1	0.6	0.6	0.7	0.6	0.5	60.0	1.0	0.7	0.6	0.8	1.1
S76054_s_at		Keratin complex 2, basic, gene 8	Krt2-8	S76054		305.6	2.3	1.0	1.3	1.2	1.7	345.3	2.8	1.6	1.6	1.4	2.0
rc_AI072634_at		Keratin complex 1, acidic, gene 18	Krt1-18	AI072634	Rn.3603	417.8	1.9	1.1	1.3	1.2	1.6	384.1	3.7	2.2	1.8	1.8	3.3
X59864mRNA_at		H19 fetal liver mRNA	H19	X59864		3.3	17.7	7.5	7.8	48.5	21.2	1.3	1.0	52.8	86.2	1.2	1.1
D31662exon#4_s_at	Signal transduction & transcription factors	Regucalcin	Rgn	D31662		865.7	0.2	0.7	1.0	0.5	0.4	863.6	0.2	1.2	0.7	0.6	0.1
rc_AA893485_at		RAB10, member RAS oncogene family	Rab10	AA893485	Rn.65864	90.1	0.5	0.5	1.3	0.7	0.7	68.0	0.3	1.3	0.6	1.0	0.4
U68544_at		Peptidylprolyl isomerase F (cyclophilin F)	Ppif	U68544	Rn.2923	276.1	0.4	0.7	0.9	0.7	1.1	399.5	0.4	0.5	0.5	0.4	1.1
rc_AA799560_at		N-myc downstream regulated gene 2	Ndrg2	AA799560	Rn.3407	799.7	0.4	1.0	1.2	0.8	0.7	1329.5	0.5	0.6	0.6	0.6	0.4
rc_AA891194_s_at		Arg/Abl-interacting protein ArgBP2	Argbp2	AA89119	Rn.24612	35.9	1.3	2.0	1.4	1.5	2.0	38.5	4.3	2.1	1.9	2.3	1.5
X57133mRNA_at		Rat mRNA for hepatocyte nuclear factor 4	HNF4	X57133		214.9	0.9	0.4	0.5	0.5	0.3	210.3	0.4	0.4	0.6	0.6	0.8
Y14933mRNA_s_at		One cut domain, family member 1	Onecut1	Y14933	Rn.48812	70.3	0.4	0.2	0.6	0.1	0.2	212.2	0.5	0.4	0.4	0.0	0.3
D86745cds_s_at		Nuclear receptor subfamily 0, group B, member 2	Nr0b2	D86745		486.2	0.2	0.9	0.6	0.4	0.2	304.0	0.5	0.4	0.5	0.7	0.6
X12752_at		CCAAT/enhancer binding protein (C/EBP), alpha	Cebpa	X12752		224.3	0.5	0.3	0.5	0.4	0.3	206.1	0.3	0.4	0.5	0.6	0.4
M81855_at	Transporters	ATP-binding cassette, sub-family B (MDR/TAP), member 1	Abcb1	M81855	Rn.82691	3.2	124.3	24.8	40.6	90.6	108.0	9.1	85.3	20.9	34.8	23.8	63.9
AB010466_s_at		ATP-binding cassette, sub-family C (CFTR/MRP), member 6	Abcc6	AB010466	Rn.29976	452.7	0.4	0.8	0.6	0.5	0.6	415.3	0.5	0.7	0.6	0.9	0.5
U53927_at		Solute carrier family 7 (cationic amino acid transporter, y+ system), member 2	Slc7a2	U53927		29.7	1.6	0.7	0.8	0.7	1.0	27.7	1.1	0.6	0.9	0.5	5.2
M77479_at		Solute carrier family 10 (sodium/bile acid cotransporter family), member 1	Slc10a1	M77479	Rn.9913	1054.3	0.2	0.8	0.7	0.4	0.4	1314.8	0.3	0.5	0.5	0.8	0.3
L23413_at		Solute carrier family 26 (sulfate transporter), member 1	Slc26a1	L23413	Rn.10016	456.1	0.5	0.7	0.8	0.5	0.6	426.3	0.6	1.1	0.6	1.2	0.7
U76379_s_at		Solute carrier family 22 (organic cation transporter), member 1	Slc22a1	U76379	Rn.11186	518.4	0.6	0.6	0.6	0.5	0.5	565.6	0.6	0.8	0.5	0.6	0.4
L27651_g_at		Solute carrier family 22 (organic anion transporter), member 7	Slc22a7	L27651	Rn.10009	423.3	0.6	0.7	0.9	0.5	0.7	477.6	0.9	1.3	0.9	1.3	0.6
U88036_at		Solute carrier organic anion transporter family, member 1a4	Slco1a4	U88036	Rn.5641	560.4	0.4	1.1	1.1	0.9	1.0	588.3	0.6	0.9	0.9	0.5	0.6

Probe ID	Functional category	Annotation	Symbol	GenBank	UniGene	Whole Liver						Isolated Hepatocytes					
						d 0	4	7	14	21	28	d 0	4	7	14	21	28
M95762_at		Solute carrier family 6 (neurotransmitter transporter, GABA), member 13	Slc6a13	M95762	Rn.10527	205.2	0.4	0.7	0.7	0.5	0.5	262.9	0.7	0.6	0.4	0.8	0.5
U28504_g_at		Solute carrier family 17 (sodium phosphate), member 1	Slc17a1	U28504	Rn.11150	36.0	0.6	1.1	1.6	2.0	2.0	78.7	0.9	1.2	1.1	1.3	0.5
M64862_at		Solute carrier organic anion transporter family, member 2a1	Slco2a1	M64862	Rn.9671	241.6	0.6	0.6	0.5	0.6	0.5	166.2	0.3	0.4	0.7	0.9	0.5
AB013112_s_at		Aquaporin 9	Aqp9	AB013112		693.4	0.6	0.7	0.8	0.7	0.7	730.2	0.9	1.2	1.2	1.6	0.5
AB005547_at		Aquaporin 8	Aqp8	AB005547	Rn.6315	158.0	1.1	0.9	0.9	0.9	1.6	103.4	1.7	2.5	2.9	3.5	2.1
AB000507_at		Aquaporin 7	Aqp7	AB000507	Rn.11111	*35.9*	1.8	1.4	1.5	1.4	1.9	13.7	4.0	4.9	5.9	5.1	4.2
rc_AA799645_g_at		FXYD domain-containing ion transport regulator 1	Fxyd1	AA799645	Rn.3828	169.6	0.4	0.6	0.5	0.5	0.4	200.9	0.5	0.3	0.3	0.5	0.5
AF080568_at	Enzymes related phospholipids	Phosphate cytidyly-ltransferase 2, ethanolamine	Pcyt2	AF080568	Rn.7291	617.9	0.4	0.4	0.8	0.6	0.7	569.7	0.6	0.8	0.6	0.8	0.6
D28560_at		Ectonucleotide pyrophosphatase/ phospho-diesterase 2	Enpp2	D28560	Rn.20403	410.9	0.1	0.9	0.8	0.6	0.4	353.6	0.4	0.7	0.7	0.8	0.5
L14441_at		Phosphatidy-lethanolamine N-methyl-transferase	Pemt	L14441	Rn.9875	758.5	0.4	0.8	0.9	0.7	0.6	809.4	0.6	0.9	0.8	1.1	0.5
rc_AA875050_at		(Ethanolamine kinase)	---	AA875050	Rn.65516	533.0	0.4	1.0	0.9	0.7	0.6	421.1	0.6	0.7	0.6	0.9	1.0
D16339_at	Protein related vitamins	Tocopherol (alpha) transfer protein	Ttpa	D16339		597.7	0.4	1.0	1.0	0.8	0.8	498.8	0.8	0.9	0.9	0.4	0.5
D14564cds_s_at		L-gulonolactone oxidase	Gulo	D14564		1483.9	0.2	0.5	0.6	0.3	0.2	1205.8	0.2	0.8	0.5	0.8	0.2
U19485_g_at	Others	Secreted phosphoprotein 2	Spp2	U19485	Rn.84	2059.5	0.6	0.8	0.8	0.7	0.6	2454.4	0.8	0.8	0.7	0.9	0.6
AF022774_g_at		Rabphilin 3A-like (without C2 domains)	Rph3al	AF022774	Rn.10986	39.9	*1.4*	*1.0*	1.5	1.7	1.5	28.5	2.4	1.9	1.2	1.7	2.9
rc_AA945050_f_at		Rat senescence marker protein 2A gene, exons 1 and 2	Smp2a	AA945050	Rn.40124	385.7	0.6	0.9	1.2	0.7	1.4	547.7	1.4	0.5	1.8	0.9	1.0
AF062389_at		Kidney-specific protein (KS)	RGD:708383	AF062389	Rn.14875	44.9	1.1	3.0	2.0	1.4	2.0	50.7	0.5	2.2	4.6	3.8	3.3
AF037072_at		Carbonic anhydrase 3	Ca3	AF037072	Rn.1647	1189.5	0.1	0.3	0.3	0.2	0.1	3196.8	0.0	0.4	0.1	0.1	*0.0*

Expression profiles of whole liver or isolated hepatocytes during fibrogenesis were obtained using a rat Genome U34A Array (Affymetrix). Marker genes for hepatocytes, which are the main contributors to the expression profile of the whole liver, are listed. Expression intensities are given for d 0, and expression intensity data for d 4, 7, 14, 21 and 28 are displayed as ratios to the d 0 expression data. Italics indicate an "absent" call by the Affymetrix software. Genes with the highest or lowest ratio in the chronic phase are shown in bold text. □ between 0.667 and 1.5; ■ ⩾ 1.5; ■ ⩽ 0.667.

different phases, such as repair in the acute phase response and fibrosis in the chronic phase response. These two groups of HSC marker genes may also be differentially regulated. Marker genes in one group may be expressed both in undifferentiated and in differentiated HSCs, while marker genes in the other group may be expressed mainly in differentiated HSCs. The functional changes associated with differentiation of HSCs during fibrosis are not clear. Schnabel *et al*[15] have temporally divided the activation process of HSCs into an initiation phase and a perpetuation phase, and HSCs in the initiation phase may play a role in wound healing[16,17] and are then eliminated by apoptosis[18,19], although some HSCs in the initiation phase differentiate into cells in the perpetuation phase. The two kinds of marker genes found in our work may be associated with the two phases.

In the present study, gene expression in HSCs *in vivo* was mostly similar to that found *in vitro*, suggesting that HSCs can be activated and produce ECM with few factors contributed by other cell types. Since HSCs also produce auto-stimulating factors such as TGF-beta, chemokines, PDGF, and IGF-1, our results strongly suggest that HSCs have self-supporting properties and few exogenous factors are required for their activation and differentiation. Even if

Table 7 Marker genes for hepatocytes that showed a strong relationship with fibrogenesis

Functional category	Cluster No.	Direction of change	Annotation	Symbol	Common
Amino acid metabolism	7	Increase	Glutamate oxaloacetate transaminase 1	Got1	J04171
	7	Increase	Glutamic-pyruvate transaminase (alanine aminotransferase)	Gpt	D10354
	9	Increase	Glutathione-S-transferase, alpha type2	Gsta2	AA945082
	3	Decrease	Cytosolic cysteine dioxygenase 1	Cdo1	AA942685
	10	Decrease	Cysteine-sulfinate decarboxylase	Csad	M64755
Cholesterol synthesis	3	Decrease	Nuclear receptor subfamily 0, group B, member 2	Nr0b2	D86745
Steroid hormone synthesis	7	Increase	Hydroxysteroid (17-beta) dehydrogenase 9	Hsd17b9	U89280
	7	Increase	(20-alpha-hydroxysteroid dehydrogenase)	---	AA866264
	1	Decrease	Hydroxysteroid (17-beta) dehydrogenase 2	Hsd17b2	X91234
	3	Decrease	Sulfotransferase, estrogen preferring	Ste	S76489
Lipid biosynthesis, metabolism, fatty acid & lipid transport	9	Increase	Carboxylesterase 2 (intestine, liver) (drug metabolism)	Ces2	AB010635
	1	Decease	2-hydroxyphytanoyl-CoA lyase(peroxisomal) alpha-oxidation	Hpcl2	AA893239
	3	Decease	Carboxylesterase 3	Ces3	L46791
	3	Decease	Hydroxyacid oxidase 2 (long chain)(peroxisomal)(alpha-oxidation)	Hao2	AI232087
	3	Decease	Fatty acid binding protein 7, brain (cytosolic)	Fabp7	U02096
	10	Decease	Alpha-methylacyl-CoA racemase Peroxisomal)	Amacr	U89905
Retinoid synthesis & metabolism	3	Decease	Retinol dehydrogenase type Ⅱ (RODH Ⅱ)	RoDHII	U33500
	10	Decease	Retinol dehydrogenase type Ⅲ	Rdh3	U18762
Dlucolysis & gluconeogenesis	7	Increase	Glucokinase	Gck	X53588
	3	Decrease	Phosphoenolpyruvate carboxykinase 1(PEPCK1) (cytosolic)	Pck1	K03243
	3	Decrease	Pyruvate carboxylase	Pc	U32314
	3	Decrease	Solute carrier family 37 (glycerol-6-phosphate transporter), member 4	Slc37a4	AF080468
	3	Decrease	Ribonuclease, RNase A family 4	Rnase4	AF041066
	3	Decrease	Ectonucleotide pyrophosphatase/phosphodiesterase 2(lysophospholipaseD)	Enpp2	D28560
Drug-metabolism	7	Increase	Cytochrome P450, family 3, subfamily a, polypeptide 13	Cyp3a13	U46118
	9	Increase	Aldehyde dehydrogenase family 1, member A1	Aldh1a1	AF001898
	1	Decrease	Cytochrome P450, family 1, subfamily a, polypeptide 2	Cyp1a2	E01184
	1	Decrease	Cytochrome P450, subfamily ⅡC (mephenytoin 4-hydroxylase)	Cyp2c	X79081
	1	Decrease	Flavin containing monooxygenase 1	Fmo1	M84719
	3	Decrease	Cytochrome P450, family 17, subfamily a, polypeptide 1	Cyp17a1	M21208
	3	Decrease	Cytochrome P450, family 3, subfamily a, polypeptide 11	Cyp3a11	M13646
	3	Decrease	Alcohol dehydrogenase 4 (class Ⅱ), pi polypeptide	Adh4	X90710
	3	Decrease	3-hydroxybutyrate dehydrogenase (heart, mitochondrial)	Bdh	AA817846
	3	Decrease	Camello-like 1 N-acetyltransferase 8, NAT8)	Cml1	AA892382
	3	Decrease	(hydroxysteroid sulfotransferase)	---	M31363
	3	Decrease	(hydroxysteroid sulfotransferase subunit)	---	AA818122
Growth factors & their receptors	7	Increase	Lipopolysaccharide binding protein	Lbp	L32132
	9	Decrease	Insulin-like growth factor binding protein 2	Igfbp2	J04486
	3	Decrease	Activin beta E	Inhbe	AF089825
	10	Decrease	Growth hormone receptor	Ghr	S49003
	10	Decrease	Insulin-like growth factor binding protein 1	Igfbp1	M58634
	10	Decrease	Deiodinase, iodothyronine, type I	Dio1	X57999
Hepatic secretory proteins	7	Increase	Alpha-fetoprotein	Afp	X02361
	1	Decrease	Alpha-2u globulin PGCL4 /// alpha-2u globulin PGCL2 /// alpha-2u-globulin (L type)/// alpha-2u globulin PGCL1 /// alpha-2u globulin PGCL3	Obp3 /// LOC298109 /// LOC298116 /// LOC259246 /// LOC259244	M27434
	1	Decrease	Alpha-2u globulin PGCL4 (Ppp2r2a protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform)	Obp3	J00738
	10	Decrease	Metallothionein	Mt1a	AI102562
Protease & protease inhibitor	9	Increase	Lipocalin 2	Lcn2	AA946503
	1	Decrease	Elastase 1, pancreatic	Ela1	L00117
	10	Decrease	Esterase 2 (liver carboxylesterase)	Es2	M20629
	3	Decrease	Serine protease inhibitor	Spin2a	D00752
Cell surface proteins & structural proteins	7	Increase	Keratin complex 2, basic, gene 8 (cytokeratin-8)	Krt2-8	S76054
	7	Increase	Similar to cytokeratin(keratin complex 1, acidic, gene 18)	(Krt1-18)	AI072634
Signal transduction	3	Decrease	Regucalcin	Rgn	D31662
Transporters	7	Increase	solute carrier family 17 (sodium phosphate), member 1	Slc17a1	U28504
	7	Increase	Aquaporin 7	Aqp7	AB000507
	9	Increase	ATP-binding cassette, sub-family B (MDR/TAP), member 1	Abcb1	M81855
	3	Decrease	Solute carrier family 10 (sodium/bile acid cotransporter family), member 1	Slc10a1	M77479

Functional category	Cluster No.	Direction of change	Annotation	Symbol	Common
Protein related vitamins	10	Decrease	L-gulono-gamma-lactone oxidase	Gulo	D14564
Others	7	Increase	Rabphilin 3A-like (without C2 domains)	Rph3al	AF022774
	1	Decrease	Carbonic anhydrase 3	Ca3	AF037072

Gene expression profiles in the chronic phase (d 7, 14, 21 and 28) were clustered into 10 patterns using K-means analysis. Clustered genes that showed a tendency to temporally decrease (clusters 1, 3 and 10) or increase (clusters 7 and 9) were selected, as shown in Table 5 in the supplemental data. Among these genes, those showing a strong relation with fibrogenesis were further selected based on a *t*-test statistical analysis of the rate of change in expression intensity and the number of days of fibrosis. Hepatocyte-specific gene markers showing a strong relation with fibrogenesis are listed.

inflammation induced by virus activation stimulates long-term or weak hepatitis, HSCs may be able to autonomously activate and promote fibrosis, and this property of HSCs may be central to promotion of fibrogenesis. Sancho-Bru *et al*[20] have recently reported that a culture model of HSCs could not exactly match the activated phenotype found in DNA microarray analysis of isolated HSCs from cirrhotic human livers, because in culture marker genes for HSCs in the perpetuation phase are predominantly expressed, relative to those in the initiation phase. However, isolation of HSCs may also alter the expression of some genes, especially the expression of genes associated with inflammation, as shown in Figures 2 and 4. Therefore, compared to studies of isolated HSCs, our approach reveals the actual behavior of HSCs *in vivo* during fibrosis.

Gpc3 has been recently proposed as a serum and histochemical marker for hepatocellular carcinoma[21,22], since it is only weakly expressed in hepatocytes in normal and cirrhotic livers. However, in our study, Gpc3 was expressed in isolated HSCs during fibrogenesis, but weakly in cultured HSCs, suggesting that HSCs require extracellular factors for expression of Gpc3 during fibrogenesis, or that Gpc3-expressing cells with abnormal characteristics may contaminate the HSC fraction. The cell type showing expression of Gpc3 requires further study.

Marker genes for inflammatory cells

In the present study, gene expression in the inflammatory cell-fraction indicated the presence of several kinds of hematopoietic cells in this fraction, leading to some uncertainty in the data from the rat fibrosis model. However, the behavior of mast cells is of note, since temporary up-regulation of mast cell markers such as chemokines and mast cell proteases indicated invasion and/or activation of mast cells around d 14. Invasion of a marked number of mast cells could not be detected with HE staining, and therefore the number of invading mast cells must be small. Mast cells not only cause acute inflammation, but also have a role in induction of chronic inflammation[12,14], and involvement of mast cells in hepatic fibrosis has been reported[12,23-25]. RANTES, a chemokine that is produced by T cells and stimulates mast cells[26,27], showed its peak expression on d 14, as shown in Figure 4. RANTES has been suggested to be a mediator of progression from acute to chronic inflammation in colitis[28], and further studies are of importance to determine whether RANTES activation of mast cells is essential for liver fibrosis.

Marker genes for hepatocytes

DNA microarray analysis of whole liver in experimental animal models of hepatic fibrosis has been reported[1,2], and characteristic behavior of hepatocyte-specific marker genes over the time course of fibrogenesis was found in this study. DNA microarray data for the whole liver are similar to those for hepatocytes, since 70% of hepatic cells are hepatocytes[8,9], and data on d 28 are generally similar to those in previous reports. However, our temporal data indicate progressive abnormal gene expression in fibrogenesis, including in the early phase of fibrogenesis. Furthermore, since our data did not contain genes expressed in other hepatic cells, the hepatocyte-specific gene set allowed examination of the molecular network in hepatocytes.

Clustered abnormalities were found in genes associated with metabolism of sulfur-containing amino acids in this study. Similar abnormalities in metabolism of sulfur-containing amino acids have been reported[29,30], and an increase in methionine concentration in blood has been found in cirrhosis[31,32], which may be related to changes in gene expression in sulfur-containing amino acid metabolism. Furthermore, S-adenosylmethionine has an important role in methylation, including the methylation of DNA. Since abnormalities likely induce tumorigenesis due to DNA instability, it is of note that long-term suppression of S-adenosylmethionine synthetase increases the risk of tumorigenesis[33]. Glycine methyltransferase also has been implicated in DNA instability[34,35], and long-term suppression of this enzyme also has an associated risk of tumorigenesis during liver fibrosis. A metabolite of methionine, homocysteine, is also suspected as a risk factor for cardiovascular disease[36,37], and an increase in the concentration of homocysteine has been found in cirrhosis[38]. Abnormal homocysteine metabolism may also have an important role in the pathogenesis of liver failure, including fatty liver, activation of HSCs (i.e., enhancement of fibrosis), cardiovascular disease, and HCC. Finally, biosynthesis of taurine from cysteine may be suppressed, possibly leading to painful muscle cramps, which are a complication of cirrhosis caused by taurine deficiency[39]. Therefore, in summary, it is apparent that abnormalities in sulfur-containing amino acid metabolism can result in development of serious diseases.

Down-regulation of enzymes related to beta-oxidation in TAA-induced experimental fibrosis has been reported[29]. Studies of the Aox knockout mouse[40] suggest that metabolites of fatty acids in beta oxidation accumulate in

the liver and stimulate PPAR-alpha, resulting in peroxisome proliferation, and tumorigenesis is a potential risk in long-term administration of PPAR-alpha agonists and in the Aox-1 knockout mouse[40,41]. Radical accumulation by blocking metabolic enzymes associated with beta-oxidation has been suggested as one explanation of tumorigenesis in Aox knockout mice, and furthermore, microarray analysis of Aox-deficient mice can show up-regulation of Lcn2, a marker of carcinogenesis[42]. Interestingly markers such as Lcn2 and Cd36 were up-regulated in hepatocytes during fibrogenesis and in the inflammatory cell fraction in the late phase in our model. Although tumorigenesis due to PPAR alpha agonist is thought not to occur in humans[41,43], abnormalities in lipid metabolism during liver fibrosis may have a role in steatofibrosis and/or enhancement of fibrosis and HCC.

Up-regulation of Gck and down-regulation of Pck1 in hepatocytes suggest a decrease in gluconeogenesis, and suppression of gluconeogenesis has been reported in cirrhosis[44]. A deficiency of Scl37a4 enzyme activity in humans causes glycogen storage disease type 1 (GSD-1) genetic disorders[45] and suppression of this molecule is associated with hepatic steatosis[46]. Abnormalities in metabolism and synthesis of sex hormone have also been found in fibrotic liver, and an increased ratio of estrogen to testosterone in serum induces feminization, which is a complication in cirrhotic males[47]. Abnormality of sex-hormone metabolism is also related to liver malignancies[48,49].

Down-regulation of Ghr is also found in hepatocytes, and long-term suppression of growth signals may greatly influence fundamental hepatic vitality and produce abnormal hepatic regeneration. Insensitivity to growth hormones in cirrhosis has been reported[50,51], and administration of growth hormone protects against experimental liver fibrosis[52]. Rgn, a regulator of calcium signaling, may have an important role in regulation of proliferation and apoptosis of hepatocytes, as well as in formation of HCC[53,54], and down-regulation of regucalcin in our model suggested a risk of HCC development. An association of Afp, Ste, Mt1a, Lcn2, Abcb1 Cml1 (Nat8) and Ca3 with hepatocellular carcinoma (HCC) formation has also been reported. Accumulation of estrogen in the liver is suspected to promote HCC[55,56], and NAT8 polymorphism may be related to HCC[57,58]. Down-regulation of metallothionein[59-61] and carbonic anhydrase[62,63], up-regulation of Mdr/Tap[64] and Lcn[42,65] occur in HCC, and proliferation of hepatoma cells is suppressed by over-expression of Rgn[53,66]. The abnormal expression of all these genes in our model is similar to that in HCC, suggesting the importance of understanding whether such changes in gene expression reflect a tumorigenic environment or even promote tumorigenesis.

Differential regulations of genes involved in key events of liver fibrosis

In addition to the expression profiles of cell type specific marker genes discussed above, we here describe how genes involved in key events of liver fibrogenesis are differentially regulated. Gene expression profiles of the whole liver for different functional categories in liver fibrogenesis, such as ECM synthesis/degradation, inflammation and oxidative stress, are shown in Figure 8. Since both synthesis and degradation of ECM occur simultaneously, both genes are put together. Figure 8 shows that most genes in each category have a common and mutually correlated expression pattern, showing different regulations for different categories. Most genes of ECM synthesis/degradation as shown in Figure 8A have a peak of up-regulation on d 4 and a following gradual up-regulation along with the progression of fibrosis, and interestingly these genes are classified into group 1 of the HSC -specific genes as shown in Figure 2. Another type of genes of ECM synthesis/degradation as shown in Figure 8B have no peak on d 4 and only a gradual up-regulation along with the progression of fibrosis, and are classified into group 2 of the HSC-specific genes as shown in Figure 2. Many genes are also involved in the inflammatory category, and here only some of them are plotted as representative in Figure 8C. Most genes of inflammation have a peak on d 4 or 7 commonly, and are classified in group 1 of the Kupffer cell fraction specfic genes as shown in Figure 4. Most genes in the catregory of oxidative stress in Figure 8D have a minimum expression on d 4, in contrast to the genes of inflammation having a peak on d 4 or 7, and are involved in hepatocyte specific genes as shown in Figure 6.

Gene expression profiles viewed from both cell types and functional categories have made more clear image on how the temporal expression pattern are closely associated in terms of both the cell specificity and functions in liver fibrosis, and are regulated differently in different categories but in a mutually correlated manner within the same category.

Pathological overview of the behavior of HSCs, inflammatory cells, and hepatocytes in fibrogenesis

Our results from gene-expression profiling using hepatic cell-specific marker genes support the hypothesis shown in Figure 9. Comparisons of gene expression in HSCs *in vivo* and *in vitro* strongly suggest that HSCs have self-supporting properties and that few exogenous molecules are required to activate and differentiate HSCs. Hepatocytes have been shown to suffer from serious stress during fibrogenesis, and signals from suppressed hepatocytes, such as radicals, prophlogistic substances and toxic metabolites due to abnormal metabolism, are able to stimulate Kupffer cells and HSCs, leading to subsequent production of HSC-stimulating-factors such as TNF-α and IL-1 by Kupffer cells. Therefore, HSCs, Kupffer cells and other inflammatory cells produce factors such as TGF-beta that suppress hepatocyte vitality and result in hepatocyte injury. Sequential activation of inflammatory cells such as lymphocytes and mast cells may be essential in this process, and even if this stimulatory circuit is small in scale during early remission of fibrogenesis, it can be maintained with appropriate stimulation even at long intervals, with small-scale inflammation induced by C-type hepatitis virus propagation. The self-supporting characteristics of HSCs may have a central role in maintenance of this circuit.

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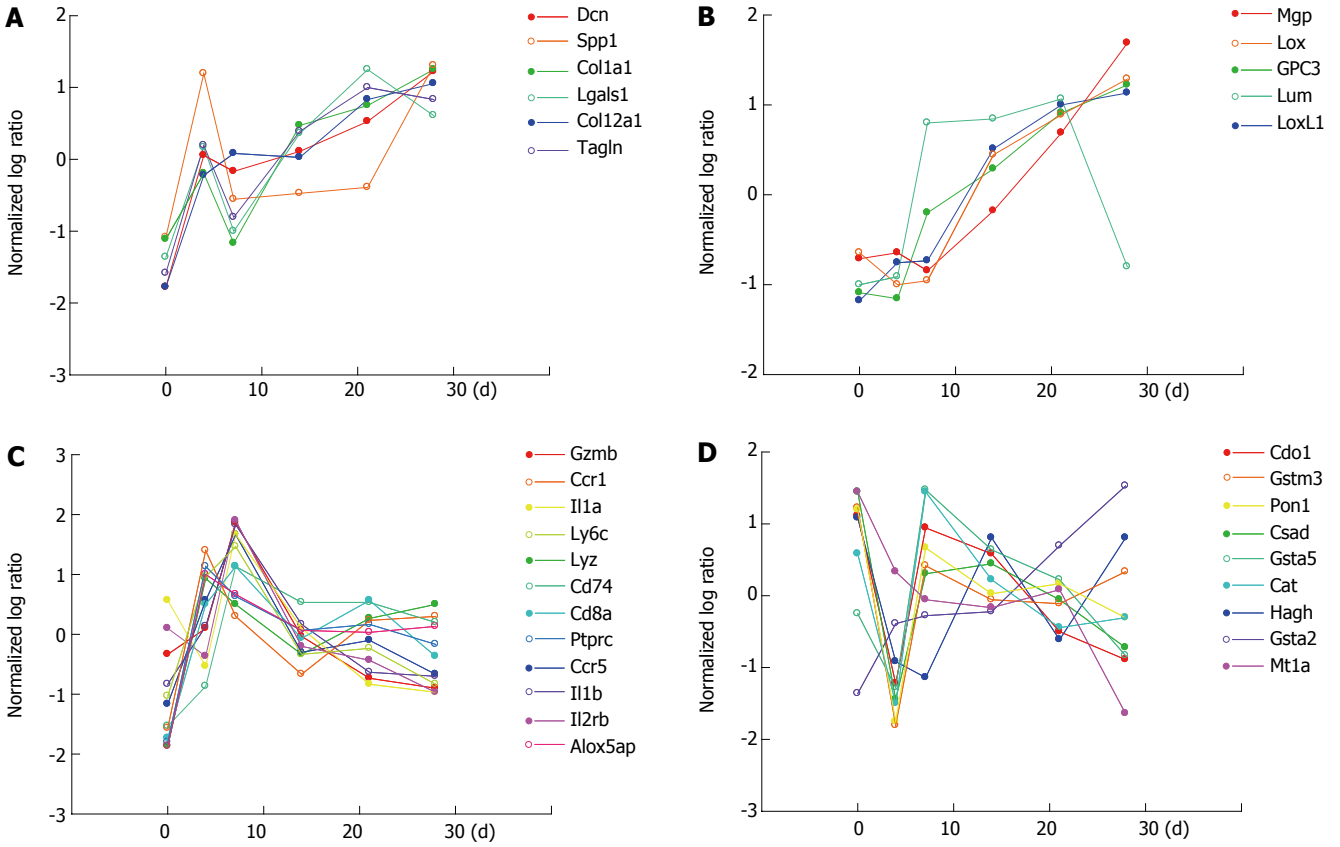

Figure 3 Differential regulations of genes involved in key events of liver fibrosis. Gene expression profiles of different events in liver fibrogenesis such as ECM synthesis/degradation, inflammation and oxidative stress, are shown. The x-axis showing the days of fibrogenesis (d 0, 4, 7 14, 21, 28) and the y-axis the normalized log ratio(scaled in terms of mean and SD, and the log base 2) of the whole liver gene expression. **A** and **B**: genes of ECM synthesis/degradation classified into group 1 (2) of HSCs as shown in Table 1; **C**: genes of inflammation involved in the Kupffer cell fraction as shown in Table 3; **D**: genes of oxidative stress involved in the hepatocytes as shown in Table 6.

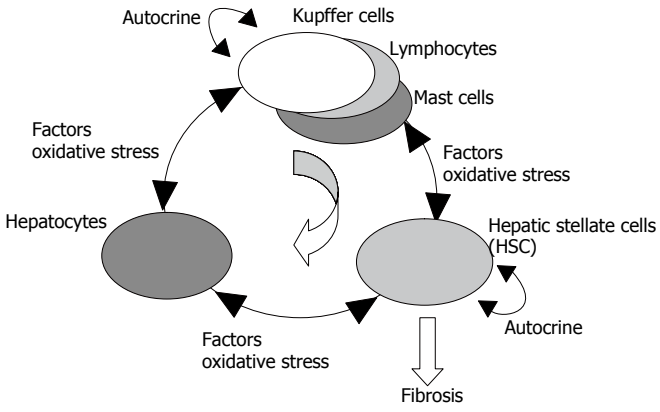

Figure 4 Circuit model of hepatic cells in fibrogenesis. Gene expression profiles show that HSCs have self-activating properties, and that widespread damage to hepatocytes occurred in development of fibrosis, suggesting a self-activating circuit model of fibrogenesis. After an initial stimulatory trigger caused by events such as virus infection, hepatic cells are able to stimulate each other, and the self-activating properties of HSCs maintain this cycle over the long term. Details are given in the text.

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