

Supplementary Appendix

Procedure S1. Subjects' eligibility and exclusion criteria

Subjects were eligible if they (i) were a male or female over 20 years of age at the time of their biopsies; (ii) reported drinking less than 21 standard drinks per week on average for men and 14 standard drinks on average per week on average for women; and (iii) had been clinically diagnosed with NAFLD according to the American Association for the Study of Liver Diseases guidelines (1).

Subjects were excluded if they had any of the following: hepatic virus infections (hepatitis B and C, cytomegalovirus, and Epstein–Barr virus), primary biliary cirrhosis, autoimmune hepatitis, sclerosing cholangitis, hemochromatosis, Wilson's disease, and drug-induced liver injury or biliary obstruction.

Procedure S2. Data collection

The diagnosis of diabetes mellitus was based on the American Diabetes Association criteria (2). Dyslipidemia was defined as serum concentrations of triglycerides ≥ 150 mg/dL and LDL cholesterol ≥ 140 mg/dL or being currently under treatment (3). Hypertension was defined as blood pressure greater than 140/90 mmHg according to the criteria of the Japanese Society of Hypertension (4) or currently under treatment. HemoglobinA1c (HbA1c) was measured by high-performance liquid chromatography assay (Automated Glycohemoglobin Analyzer HLC-723®G11; Tosoh Inc., Tokyo, Japan). Fasting plasma glucose (FPG) levels were measured by the glucose oxidase immobilized enzyme membrane electrode method (GA08 III ; A&T Corporation., Kanagawa, Japan). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltransferase (GGT) levels were measured by JSCC transferable method (LABOSPECT008α; Hitachi, Ltd. Tokyo, Japan). Serum total cholesterol, triglyceride, and HDL cholesterol levels were measured enzymatically (LABOSPECT008α; Hitachi, Ltd. Tokyo, Japan). All tests were conducted and analyzed

at the central clinical laboratory in our hospital.

Procedure S3. FIB-4 index

The FIB-4 index is a noninvasive tool (i.e., $\text{FIB-4 index} = \text{age}[\text{years}] \times \text{aspartate aminotransferase}[\text{U/L}] / [\text{platelet count}[10^9/\text{L}] \times (\text{alanine aminotransferase}[\text{U/L}])^{1/2}]$) for assessing hepatic fibrosis(5)(6). The FIB-4 index is easy to use in clinical practice and has a comparable diagnostic capability for advanced fibrosis to magnetic resonance elastography (7).

Procedure S4. Hepatic gene expression

RNA-sequencing (RNA-seq) was performed by using SMART-Seq® Stranded Kit (Takara Bio, Inc., Japan) and NovaSeq 6000 Sequencing System (Illumina, Inc., USA). Expression data were processed by BRB-ArrayTools (<https://brb.nci.nih.gov/BRB-ArrayTools>). The quality of the isolated RNA was estimated after electrophoresis using an Agilent 2100 Bioanalyzer (Palo Alto, CA). Pair-wised comparisons were performed to compare the gene expression between the first and second liver biopsied samples using edgeR with the generalized linear model likelihood ratio test approach (BRB-array tool). Functional ontology enrichment analysis was conducted to compare the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. To address which components of resident cells participate in alleviating liver fibrosis and glycemic control, we performed gene set enrichment analyses using representative gene sets associated with resident cells in the human liver defined by single-cell RNA-Seq analyses (8). Liver resident cells were clustered into hepatocytes 1, 2, 3, 4, 5, and 6, cholangiocytes, central liver sinusoidal endothelial cell (LSEC), periportal LSEC, portal endothelial cells, stellate cells, inflammatory MACS, non-inflammatory MACS, $\alpha\beta$ T cells, $\gamma\delta$ T cells-1 and -2, NK cells, mature B cells, plasma cells, and erythroid cells (8). Functional ontology

enrichment analyses were conducted to compare each cell component distribution of the differentially expressed genes.

Procedure S5. Statistical analysis

A generalized linear mixed model is useful for settings where repeated measurements are made for the same subject during longitudinal observations and handling missing or volatile parameters. Therefore, it is suitable for continuous liver biopsy studies in real-world clinical practice.

For statistical analysis, the Jonckheere–Terpstra trend test for continuous variables and the Cochran–Armitage trend test for categorical variables was used to examine associations between baseline variables and final hepatic pathology. Associations between changes in clinical parameters and the progression of liver pathology over time were examined using generalized linear mixed models adjusted for covariates.

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Supplemental Table 1 Baseline clinical characteristics in NAFLD subjects with or without type 2 diabetes

	Total (n = 118)	Type 2 diabetes (n = 86)	Non-diabetes (n = 32)	p†
Age (years)	50.7 (14.1)	52.9 (13.5)	44.5 (14.3)	0.004
Gender (Male : Female)	67 : 51	48 : 38	19 : 13	0.728
Duration of Type 2 diabetes (year)		5.44 (7.34)		
Hypertension, n (%)	53 (44.9)	42 (48.8)	11 (34.4)	0.160
Dyslipidemia, n (%)	86 (72.9)	67 (77.9)	19 (59.4)	0.044
Weight (kg)	76.1 (18.0)	76.0 (18.3)	76.3 (17.5)	0.943
Body-mass index (kg/m ²)	28.8 (5.6)	28.8 (5.9)	28.9 (5.0)	0.676
Aspartate aminotransferase (IU/L)	46.42 (39.1)	46.48 (42.4)	46.28 (29.2)	0.995
Alanine aminotransferase (IU/L)	68.1 (48.3)	68.7 (50.7)	66.5 (41.9)	0.961
Gamma-glutamyltransferase (IU/L)	74.78 (51.9)	78.8 (56.3)	63.97 (36.3)	0.322
HbA1c (%)	7.6 (2.0)	8.1 (2.0)	5.7 (0.5)	<0.001
Fasting plasma glucose (mg/dL)	129.2 (44.7)	141.0 (46.5)	96.2 (7.6)	<0.001
Platelets (x10 ⁹ /L)	222.6 (66.0)	221.5 (61.4)	225.7 (78.1)	0.714
Total cholesterol (mg/dL)	193.6 (44.5)	190.6 (42.5)	201.6 (49.6)	0.244
Triglyceride (mg/dL)	149.2 (92.8)	159.1 (96.4)	120.5 (76.0)	0.009
HDL cholesterol (mg/dL)	46.37 (12.0)	46.22 (12.2)	46.85 (11.6)	0.857
Liver fibrosis stage, n (%)				0.863
0	10 (8.5)	8 (9.3)	2 (6.3)	
1	52 (44.1)	37 (43.0)	15 (46.9)	

2	25 (21.2)	16 (18.6)	9 (28.1)	
3	24 (20.3)	21 (24.4)	3 (9.4)	
4	7 (5.9)	4 (4.7)	3 (9.4)	
Steatosis stage, n (%)				0.731
0	0 (0)	0 (0)	0 (0)	
1	53 (44.9)	37 (43.0)	16 (50.0)	
2	42 (35.6)	33 (38.4)	9 (28.1)	
3	23 (19.5)	16 (18.6)	7 (21.9)	
Lobular inflammation stage, n(%)				0.184
0	2 (1.7)	0 (0)	2 (6.3)	
1	38 (32.2)	28 (32.6)	10 (31.3)	
2	64 (54.2)	45 (52.3)	19 (59.4)	
3	1 (3.1)	13 (15.1)	14 (11.9)	
Hepatocellular ballooning stage, n(%)				0.193
0	26 (22)	16 (18.6)	10 (31.3)	
1	52 (44.1)	39 (45.3)	13 (40.6)	
2	40 (33.9)	31 (36)	9 (28.1)	
NAFLD activity score	4.63 (1.56)	4.76 (0.16)	4.28 (0.32)	0.231
NAFL, n (%)	26 (22.0)	16 (18.6)	10 (31.3)	
NASH, n (%)	92 (78.0)	70 (81.4)	22 (68.8)	
Treatment, n (%)				

Insulin		14 (16.3)		
Sulfonylureas		18 (20.9)		
Metformin		17 (19.8)		
αGI		17 (19.8)		
DPP4i		9 (10.5)		
GLP-1 RA		3 (3.5)		
Glinide		5 (5.8)		
TZD		5 (5.8)		
SGLT2 inhibitor		0 (0)		
Ursodeoxycholic acid	3 (2.5)	2 (2.3)	1 (3.1)	0.806

Data are mean (SD) or n (%) unless otherwise stated.

†The between-group comparison at baseline was performed with the χ^2 test or Fisher test for categorical variables and the Mann-Whitney U test in nonparametric parameters or the two-sample t test in normal distribution for continuous parameters.

Supplemental Table 2 Generalized linear mixed model for fibrosis scores by type 2 diabetes and HbA1c adjusted with gender, age, and BMI

	Total		
	Standardized Coefficients	95%CI	p-value
Number of liver biopsies	-0.13	(-0.236–0.030)	0.011
Gender	-0.02	(-0.197–0.159)	0.834
Age (years)	0.37	(0.179–0.553)	0.000
Body-mass index (kg/m ²)	0.28	(0.113–0.450)	0.001
Type 2 diabetes	-0.52	(-1.116–0.082)	0.091
Type 2 diabetes ×HbA1c	0.06	(-0.001–0.126)	0.054

Supplemental Table 3a Baseline clinical characteristics in NAFLD subjects for hepatic gene expression analyses

	Total (n = 33)	Type 2 diabetes (n = 28)	Non-diabetes (n = 5)	p†
Age (years)	49.0 (13.4)	50.8 (12.5)	39 (15.1)	0.066
Gender (Male : Female)	13:20	12:16	1:4	0.335
Duration of Type 2 diabetes (year)		3.9 (4.4)		
Hypertension, n (%)	17 (51.5)	15 (53.6)	2 (40)	0.576
Dyslipidemia, n (%)	27 (81.8)	24 (85.7)	3 (60)	0.170
Weight (kg)	78.0 (21.9)	79.1 (23.2)	72.2 (12.2)	0.524
Body-mass index (kg/m ²)	29.7 (7.4)	29.8 (7.9)	28.9 (3.4)	0.575
Aspartate aminotransferase (IU/L)	38.1 (20.8)	37.2 (18.5)	43.2 (33.5)	1.000
Alanine aminotransferase (IU/L)	61.8 (36.4)	60.4 (34.9)	69.6 (48.3)	0.715
Gamma-glutamyltransferase (IU/L)	58.9 (34.4)	58.8 (34.8)	59.2 (35.4)	0.903
HbA1c (%)	7.9 (2.4)	8.2 (2.4)	5.7 (0.3)	0.002
Fasting plasma glucose (mg/dL)	129.3 (38.6)	134.4 (38.7)	94.5 (7.8)	0.008
Platelets (x10 ⁹ /L)	233.2 (58.0)	227.4 (57.4)	265.2 (56.6)	0.247
Total cholesterol (mg/dL)	171.8 (52.6)	170.9 (53.5)	177.8 (53.3)	0.848
Triglyceride (mg/dL)	138.9 (76.5)	147.9 (76.7)	76.3 (38.2)	0.034
HDL cholesterol (mg/dL)	47.9 (26.9)	47.5 (28.6)	50.3 (9.8)	0.230
Liver fibrosis stage, n (%)				0.391
0	5 (15.2)	5 (17.9)	0 (0)	
1	16 (48.5)	11 (39.3)	5 (100)	

2	3 (9.1)	3 (10.7)	0 (0)	
3	9 (27.3)	9 (32.1)	0 (0)	
4	0 (0)	0 (0)	0 (0)	
Steatosis stage, n (%)				0.268
0	0 (0)	0 (0)	0 (0)	
1	12 (36.4)	11 (39.3)	1 (20.0)	
2	15 (45.5)	13 (46.4)	2 (40.0)	
3	6 (18.2)	4 (14.3)	2 (40.0)	
Lobular inflammation stage, n(%)				0.715
0	0 (0)	0 (0)	0 (0)	
1	8 (24.2)	8 (28.6)	0 (0)	
2	20 (60.6)	15 (53.6)	5 (100)	
3	5 (15.2)	5 (17.9)	0 (0)	
Hepatocellular ballooning stage, n(%)				0.542
0	8 (24.2)	6 (21.4)	2 (40.0)	
1	17 (51.5)	15 (53.6)	2 (40.0)	
2	8 (24.2)	7 (25.0)	1 (20.0)	
NAFLD activity score	4.73 (1.61)	4.68 (0.31)	5.00 (0.71)	0.715
NAFL, n (%)	8 (24.2)	6 (21.4)	2 (40.0)	
NASH, n (%)	25 (75.8)	22 (78.5)	3 (60.0)	
Treatment, n (%)				

Insulin		7 (25.0)	
Sulfonylureas		6 (21.4)	
Metformin		7 (25.0)	
αGI		4 (14.3)	
DPP4i		7 (25.0)	
GLP-1 RA		1 (3.6)	
Glinide		1 (3.6)	
TZD		1 (3.6)	
SGLT2 inhibitor		0 (0)	
Ursodeoxycholic acid	0 (0)	0 (0)	0 (0)

Data are mean (SD) or n (%) unless otherwise stated.

†The between-group comparison at baseline was performed with the χ^2 test or Fisher test for categorical variables and the Mann-Whitney U test in nonparametric parameters or the two-sample t test in normal distribution for continuous parameters.

Supplemental Table 3b Baseline clinical characteristics divided into 3 groups by fibrosis score in NAFLD subjects for hepatic gene expression analyses

	Fibrosis progression (n = 6)	Fibrosis unchanged (n = 20)	Fibrosis regression (n = 7)	<i>p</i> †††
Age (years)	40.5 (6.4)	52.6 (14.5)	46.3 (11.6)	0.129
Gender (Male : Female)	1:5	9:11	3:4	0.461
Type 2 diabetes, n (%)	5 (83.3)	16 (80.0)	7 (100)	0.454
Duration of Type 2 diabetes (year)	3.7 (4.3)	4.3 (4.9)	3.1 (3.7)	0.940
Hypertension, n (%)	3 (50.0)	11 (55.0)	3 (42.9)	0.859
Dyslipidemia, n (%)	3 (50.0)	18 (90.0)	6 (85.7)	0.086
Weight (kg)	85.3 (28.1)	72.4 (16.5)	88.0 (27.4)	0.181
Body-mass index (kg/m ²)	32.3 (7.6)	27.6 (3.9)	33.4 (12.6)	0.267
Aspartate aminotransferase (IU/L)	49.2 (34.9)	35.4 (18.2)	36.6 (9.7)	0.772
Alanine aminotransferase (IU/L)	82.7 (55.8)	55.8 (32.5)	60.9 (24.0)	0.493
Gamma-glutamyltransferase (IU/L)	57.7 (27.3)	50.1 (28.7)	85 (44.9)	0.117
HbA1c (%)	8.0 (1.9)	7.7 (2.7)	8.3 (1.8)	0.414
Fasting plasma glucose (mg/dL)	136.2 (46.1)	123.8 (39.8)	139.5 (28.7)	0.397
Platelets (x10 ⁹ /L)	285.5 (55.6)	210.1 (55.5)	254.3 (24.2)	0.013
Total cholesterol (mg/dL)	176.8 (52.2)	174.9 (44.4)	158.9 (76.7)	0.979
Triglyceride (mg/dL)	115.0 (77.5)	137.1 (83.8)	164.6 (53.1)	0.197
HDL cholesterol (mg/dL)	70.8 (56.3)	42.5 (10.9)	42.7 (9.6)	0.238
Liver fibrosis stage, n (%)				0.106
0	2 (33.3)	2 (10.0)	1 (14.3)	
1	4 (66.7)	10 (50.0)	2 (28.6)	

	2	0 (0)	1 (5.0)	2 (28.6)
	3	0 (0)	7 (35.0)	2 (28.6)
	4	0 (0)	0 (0)	0 (0)
NAFL, n (%)		0 (0)	6 (30.0)	2 (28.6)
NASH, n (%)		6 (100)	14 (70.0)	5 (71.4)
Treatment, n (%)				
Insulin		1 (16.7)	4 (20.0)	2 (28.6)
Sulfonylureas		0 (0)	4 (20.0)	2 (28.6)
Metformin		0 (0)	5 (25.0)	2 (28.6)
αGI		2 (33.3)	1 (5.0)	1 (14.3)
DPP4i		0 (0)	6 (30.0)	1 (14.3)
GLP-1 RA		0 (0)	1 (5.0)	0 (0)
Glinide		1 (16.7)	0 (0)	0 (0)
TZD		0 (0)	1 (5.0)	0 (0)
SGLT2 inhibitor		0 (0)	0 (0)	0 (0)
Ursodeoxycholic acid		0 (0)	0 (0)	0 (0)

Data are mean (SD) or n (%) unless otherwise stated.

†††The ANOVA in nonparametric parameters or Kruskal– Wallis test in normal distribution for continuous parameters

Supplemental Table 3c Baseline clinical characteristics divided into 3 groups by HbA1c score in NAFLD subjects for hepatic gene expression analyses

	HbA1c elevation (n = 9)	HbA1c unchanged (n = 8)	HbA1c reduction (n = 16)	<i>p</i>
Age (years)	43.2 (10.4)	54.4 (17.3)	49.6 (12.2)	0.265
Gender (Male : Female)	4:5	2:6	7:9	0.641
Type 2 diabetes, n (%)	7 (77.8)	5 (62.5)	16 (100)	0.047
Duration of Type 2 diabetes (year)	8.4 (5.8)	2.1 (1.9)	2.6 (2.9)	0.022
Hypertension, n (%)	5 (55.6)	4 (50.0)	8 (50.0)	0.962
Dyslipidemia, n (%)	9 (100)	5 (62.5)	13 (81.3)	0.143
Weight (kg)	74.9 (15.7)	70.6 (12)	83.5 (27.5)	0.359
Body-mass index (kg/m ²)	28.0 (4.1)	28.0 (4.1)	31.4 (9.7)	0.994
Aspartate aminotransferase (IU/L)	31.2 (12.5)	50.9 (29.6)	35.6 (17.6)	0.300
Alanine aminotransferase (IU/L)	58.6 (36.4)	73.9 (37.6)	57.5 (36.9)	0.436
Gamma-glutamyltransferase (IU/L)	59.1 (36.1)	61.9 (31.1)	57.3 (37.0)	0.880
HbA1c (%)	6.7 (0.9)	6.2 (0.8)	9.2 (2.7)	0.004
Fasting plasma glucose (mg/dL)	110.2 (22.7)	111.2 (18.5)	146.8 (43.8)	0.017
Platelets (x10 ⁹ /L)	242.3 (53.8)	222.5 (68.2)	233.3 (58)	0.822
Total cholesterol (mg/dL)	157.8 (45.1)	180.9 (39.1)	175.7 (62.1)	0.479
Triglyceride (mg/dL)	121.4 (35.1)	123.6 (72.3)	155.5 (93.5)	0.735
HDL cholesterol (mg/dL)	44.3 (11.0)	46.7 (9.6)	50.4 (37.1)	0.549
Liver fibrosis stage, n (%)				0.371
0	3 (33.3)	1 (12.5)	1 (6.3)	
1	3 (33.3)	5 (62.5)	8 (50.0)	

	2	1 (11.1)	1 (12.5)	1 (6.3)
	3	2 (22.2)	1 (12.5)	6 (37.5)
	4	0 (0)	0 (0)	0 (0)
NAFL, n (%)		4 (44.4)	2 (25.0)	2 (12.5)
NASH, n (%)		5 (55.5)	6 (75.0)	14 (87.6)
Treatment, n (%)				
Insulin		2 (22.2)	1 (12.5)	4 (25.0)
Sulfonylureas		4 (44.4)	1 (12.5)	1 (6.3)
Metformin		5 (55.6)	1 (12.5)	1 (6.3)
αGI		1 (11.1)	1 (12.5)	2 (12.5)
DPP4i		4 (44.4)	2 (25.0)	1 (6.3)
GLP-1 RA		1 (11.1)	0 (0)	0 (0)
Glinide		1 (11.1)	0 (0)	0 (0)
TZD		1 (11.1)	0 (0)	0 (0)
SGLT2 inhibitor		0 (0)	0 (0)	0 (0)
Ursodeoxycholic acid		0 (0)	0 (0)	0 (0)

Data are mean (SD) or n (%) unless otherwise stated.

†††The ANOVA in nonparametric parameters or Kruskal–Wallis test in normal distribution for continuous parameters

Supplemental Table 4 Generalized linear mixed model for fibrosis score in NAFLD subjects for hepatic gene expression analyses

	Total			Type 2 diabetes mellitus		
	Standardized Coefficients	95%CI	p-value	Standardized Coefficients	95%CI	p-value
Number of liver biopsies	-0.09	(-0.784–0.61)	0.802	-0.07	(-0.831–0.69)	0.851
Gender	-0.02	(-0.321–0.277)	0.883	-0.01	(-0.335–0.323)	0.971
Age (years)	-0.34	(-0.723–0.036)	0.075	-0.32	(-0.739–0.105)	0.136
Observation period (day)	-0.01	(-0.831–0.813)	0.982	-0.11	(-0.995–0.784)	0.811
Body-mass index (kg/m ²)	0.48	(0.226–0.735)	<0.001	0.50	(0.195–0.797)	0.002
Aspartate aminotransferase (IU/L)	0.50	(0.072–0.935)	0.023	0.45	(-0.044–0.94)	0.073
Alanine aminotransferase (IU/L)	-0.89	(-1.402–-0.368)	0.001	-0.75	(-1.28–-0.225)	0.007
Gamma-glutamyltransferase (IU/L)	0.37	(0.041–0.701)	0.029	0.30	(0.005–0.591)	0.047
Fasting plasma glucose (mg/dL)	-0.14	(-0.394–0.116)	0.278	-0.19	(-0.480–0.105)	0.200
HbA1c (%)	0.13	(-0.023–0.292)	0.091	0.15	(-0.028–0.327)	0.095
Platelets (x10 ⁹ /L)	-0.69	(-0.973–-0.415)	<0.001	-0.75	(-1.07–-0.421)	<0.001
Total cholesterol (mg/dL)	0.23	(-0.103–0.561)	0.171	0.30	(-0.077–0.676)	0.115
Triglyceride (mg/dL)	-0.08	(-0.301–0.151)	0.506	-0.08	(-0.336–0.179)	0.540
HDL cholesterol (mg/dL)	-0.34	(-0.625–-0.057)	0.020	-0.43	(-0.767–-0.096)	0.013
Treatment						

Insulin	0.05	(-0.239–0.329)	0.749	0.03	(-0.303–0.36)	0.863
Metformin	-0.03	(-0.305–0.254)	0.855	0.03	(-0.304–0.362)	0.858
Sulfonylureas	0.15	(-0.151–0.444)	0.325	0.13	(-0.229–0.49)	0.465
Ursodeoxycholic acid	0.08	(-0.333–0.498)	0.690	0.09	(-0.243–0.417)	0.594

Supplemental Table 5 Differential signaling pathways in the liver of NAFLD participants with fibrosis progression

KEGG Pathway	Pathway description	Number of genes	LS permutation p-value	KS permutation p-value	Efron-Tibshirani's GSA test p-value	Up/Do wn
hsa00071	Fatty acid degradation	42	0.00001	0.00001	0.005 (+)	Down
hsa00280	Valine, leucine and isoleucine degradation	44	0.00001	0.00001	0.005 (+)	Down
hsa04610	Complement and coagulation cascades	69	0.00001	0.00001	0.025 (+)	Down
hsa00100	Steroid biosynthesis	18	0.0001	0.00001	< 0.005 (+)	Down
hsa00640	Propanoate metabolism	31	0.00031	0.00001	0.005 (+)	Down
hsa04146	Peroxisome	80	0.00048	0.00001	0.065 (+)	Down
hsa00982	Drug metabolism - cytochrome P450	64	0.00066	0.00001	0.055 (+)	Down
hsa00650	Butanoate metabolism	28	0.00139	0.00065	0.005 (+)	Down
hsa00830	Retinol metabolism	57	0.00159	0.00001	0.075 (+)	Down
hsa00900	Terpenoid backbone biosynthesis	15	0.00239	0.00382	0.015 (+)	Down
hsa00620	Pyruvate metabolism	37	0.00315	0.00044	0.04 (+)	Down
hsa00010	Glycolysis / Gluconeogenesis	62	0.00315	0.00002	0.01 (+)	Down
hsa00330	Arginine and proline metabolism	51	0.0034	0.02942	0.075 (+)	Down
hsa00020	Citrate cycle (TCA cycle)	30	0.00367	0.00001	0.01 (+)	Down
hsa00250	Alanine, aspartate and glutamate metabolism	33	0.00455	0.0335	0.01 (+)	Down
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	64	0.00471	0.0004	0.035 (-)	UP

hsa05211	Renal cell carcinoma	70	0.00636	0.00251	0.415 (+)	Down
hsa00980	Metabolism of xenobiotics by cytochrome P450	63	0.00641	0.00003	0.1 (+)	Down
hsa05221	Acute myeloid leukemia	56	0.0068	0.00198	0.475 (-)	UP
hsa00630	Glyoxylate and dicarboxylate metabolism	17	0.00736	0.00044	0.015 (+)	Down
hsa00310	Lysine degradation	46	0.01348	0.00014	0.125 (+)	Down
hsa00380	Tryptophan metabolism	45	0.01465	0.00073	0.06 (+)	Down
hsa04660	T cell receptor signaling pathway	99	0.01599	0.00339	0.05 (-)	UP
hsa00410	beta-Alanine metabolism	22	0.0164	0.00055	0.025 (+)	Down
hsa00120	Primary bile acid biosynthesis	15	0.01684	0.00199	0.025 (+)	Down
hsa00053	Ascorbate and aldarate metabolism	20	0.01756	0.00006	0.01 (+)	Down
hsa00260	Glycine, serine and threonine metabolism	32	0.01791	0.00397	0.14 (+)	Down
hsa03320	PPAR signaling pathway	64	0.01914	0.004	0.145 (+)	Down
hsa00750	Vitamin B6 metabolism	6	0.0199	0.00382	0.005 (+)	Down
hsa05142	Chagas disease (American trypanosomiasis)	95	0.02658	0.00022	0.22 (-)	UP
hsa00040	Pentose and glucuronate interconversions	28	0.03063	0.00115	< 0.005 (+)	Down
hsa04912	GnRH signaling pathway	87	0.03433	0.00019	0.04 (-)	UP
hsa03440	Homologous recombination	28	0.03812	0.1554	< 0.005 (-)	UP

hsa00860	Porphyrin and chlorophyll metabolism	39	0.04496	0.0002	0.14 (+)	Down
hsa00983	Drug metabolism - other enzymes	47	0.04674	0.00313	0.105 (+)	Down
hsa04370	VEGF signaling pathway	68	0.04699	0.00129	< 0.005 (-)	UP
hsa04012	ErbB signaling pathway	87	0.059	0.00396	0.21 (-)	UP
hsa00270	Cysteine and methionine metabolism	36	0.10414	0.00422	0.175 (+)	Down
hsa05110	Vibrio cholerae infection	51	0.19219	0.00315	0.225 (+)	Down
hsa03050	Proteasome	42	0.29756	0.00001	0.24 (+)	Down
hsa03010	Ribosome	86	0.92531	0.00159	0.255 (+)	Down

To address the molecular basis underlying the liver fibrosis progression, we examined serial hepatic gene expression profiles associated with liver fibrosis using RNA-Seq in 33 subjects. Baseline clinical characteristics in subjects for hepatic gene expression analyses are described in Supplementary Table 1.

Pathway analyses indicated that genes involved in fatty acid degradation, amino acids catabolism, glycolysis/gluconeogenesis, citrate cycle, drug, and xenobiotics metabolism were coordinately downregulated, whereas genes involved in the T cell receptor signaling pathway, the ErbB signaling pathway, and the VEGF signaling pathway were coordinately upregulated in the liver of subjects with hepatic fibrosis progression during the course.

Coordinated upregulation of genes involved in the T cell receptor signaling pathway and the ErbB signaling pathway suggests inflammation and hepatocellular injury leading to liver fibrosis. The epidermal growth factor receptor (EGFR) and its interactive signaling partner ErbB family of receptor tyrosine kinases have been implicated in liver fibrosis (Ref. 1). Activated hepatic stellate cells secrete vascular endothelial growth factor (VEGF) that plays a vital role in the development of liver fibrosis (Ref. 2). In the present study, genes involved in the VEGF signaling pathway were coordinately upregulated in association with fibrosis progression, which may be causal for hepatic stellate cell activation into myofibroblasts.

References.

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- Ref. 2. Corpechot C, Barbu V, Wendum D, Kinnman N, Rey C, Poupon R, Housset C, Rosmorduc O: Hypoxia-induced VEGF and collagen I expressions are associated with angiogenesis and fibrogenesis in experimental cirrhosis. Hepatology 2002;35:1010-1021

Supplemental Table 6 Differential signaling pathways in the liver of NAFLD participants with HbA1c elevation

KEGG Pathway	Pathway description	Number of genes	LS permutation p-value	KS permutation p-value	Efron-Tibshirani's test p-value	GSA	Up/Down
hsa03050	Proteasome	42	0.00001	0.00001	0.015 (-)		Up
hsa05130	Pathogenic Escherichia coli infection	53	0.00001	0.04826	0.02 (-)		Up
hsa04740	Olfactory transduction	68	0.00005	0.00001	< 0.005 (+)		Down
hsa00010	Glycolysis / Gluconeogenesis	62	0.00073	0.0133	0.015 (-)		Up
hsa00020	Citrate cycle (TCA cycle)	30	0.00112	0.03017	< 0.005 (-)		Up
hsa03030	DNA replication	36	0.00144	0.01403	0.09 (-)		Up
hsa04966	Collecting duct acid secretion	25	0.00202	0.00298	0.005 (-)		Up
hsa00620	Pyruvate metabolism	37	0.00328	0.04405	< 0.005 (-)		Up
hsa00512	Mucin type O-Glycan biosynthesis	28	0.02207	0.00037	0.015 (+)		Down
hsa00062	Fatty acid elongation	8	0.02815	0.00195	0.005 (-)		Up
hsa04742	Taste transduction	24	0.04657	0.09645	< 0.005 (+)		Down
hsa04730	Long-term depression	63	0.0718	0.09689	< 0.005 (+)		Down
hsa03450	Non-homologous end-joining	13	0.09222	0.00153	0.27 (+)		Down
hsa04970	Salivary secretion	68	0.2263	0.07484	< 0.005 (+)		Down
hsa04070	Phosphatidylinositol signaling system	77	0.24415	0.14145	< 0.005 (+)		Down
hsa05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	72	0.36299	0.21378	< 0.005 (+)		Down

To address the molecular basis underlying the liver fibrosis progression, we examined serial hepatic gene expression profiles associated with glycemic control using RNA-Seq in 33 subjects. Baseline clinical characteristics in subjects for hepatic gene expression analyses are described

in Supplementary Table 1.

Pathway analyses indicated that genes involved in the proteasome, glycolysis/gluconeogenesis, pyruvate metabolism, citrate cycle, fatty acid elongation, phosphatidylinositol signaling system, pathogenic *E. coli* infection, and DNA replication are coordinately upregulated in the liver of subjects with HbA1c elevation during the course. These alterations may be the cause and consequence of inadequately controlled diabetes.

