Host Cell Responses Induced by Hepatitis C Virus Binding

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Initiation of hepatitis C virus (HCV) infection is mediated by docking of the viral envelope to the hepatocyte cell surface membrane followed by entry of the virus into the host cell. Aiming to elucidate the impact of this interaction on host cell biology, we performed a genomic analysis of the host cell response following binding of HCV to cell surface proteins. As ligands for HCV–host cell surface interaction, we used recombinant envelope glycoproteins and HCV-like particles (HCV-LPs) recently shown to bind or enter hepatocytes and human hepatoma cells. Gene expression profiling of HepG2 hepatoma cells following binding of E1/E2, HCV-LPs, and liver tissue samples from HCV-infected individuals was performed using a 7.5-kd human cDNA microarray. Cellular binding of HCV-LPs to hepatoma cells resulted in differential expression of 565 out of 7,419 host cell genes. Examination of transcriptional changes revealed a broad and complex transcriptional program induced by ligand binding to target cells. Expression of several genes important for innate immune responses and lipid metabolism was significantly modulated by ligand–cell surface interaction. To assess the functional relevance and biological significance of these findings for viral infection *in vivo*, transcriptional changes were compared with gene expression profiles in liver tissue samples from HCV-infected patients or controls. Side-by-side analysis revealed that the expression of 27 genes was similarly altered following HCV-LP binding in hepatoma cells and viral infection *in vivo*. In conclusion, HCV binding results in a cascade of intracellular signals modulating target gene expression and contributing to host cell responses *in vivo*. Reprogramming of cellular gene expression induced by HCV–cell surface interaction may be part of the viral strategy to condition viral entry and replication and escape from innate host cell responses. *Supplementary material for this article can be found on the HEPATOLOGY website (http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html).* (HEPATOLOGY 2006;43: 1326-1336.)

Initiation of hepatitis C virus (HCV) infection is mediated by docking of the viral envelope to the hepatocyte cell surface membrane followed by entry of the virus into the host cell. Several lines of evidence have demonstrated that binding and entry of HCV is mediated by the HCV envelope glycoproteins E1 and E2.[1-4] Host cell proteins implicated to mediate these very first steps of virus–host interaction include CD81,[2-6] the LDL receptor,[7] scavenger receptor BI,[8,9] and highly sulfated heparan sulfate.[10]

In recent years, it has become clear that the information exchange between incoming viruses and the host cell during the first steps of virus–host interaction is not limited to the cues given to the virus by the cell resulting in cellular binding and entry of the virus.[11] For many viruses, virus–host interaction resembles a two-way dialogue in which the virus takes advantage of the cell's own signal transduction systems to transmit signals to the cells. These signals, which are usually generated at the cell surface, induce changes that facilitate entry, prepare the cells for invasion, and neutralize host defenses.[11]

As a well-characterized example, human cytomegalovirus activates several signaling pathways through the interaction between envelope glycoprotein B and epidermal

Abbreviations: HCV, hepatitis C virus; HCV-LPs, HCV-like particles; cDNA, complementary DNA; RT-PCR, reverse-transcriptase polymerase chain reaction; PCR, polymerase chain reaction.

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growth-factor receptor.[12] HIV uses chemokine receptor 5 on $CD4^+$ T cells to transmit a signal-inducing chemotaxis of T cells.[13,14]

The effect of HCV envelope–cell surface protein interaction on target cell functions is unknown. Using a genomic analysis of responses to HCV-like particles (HCV-LPs)[10,15] binding to hepatoma cells, we demonstrated that binding of HCV envelope glycoproteins to host cells results in a cascade of intracellular signals modulating cellular gene expression, which may condition the cell for support of viral propagation.

Materials and Methods

Cellular Binding of HCV-LPs to Target Cells and Isolation of RNA. HepG2 cells were incubated with HCV-LPs (corresponding to 0.5 μg/mL HCV-LP E2 as determined via ELISA[16,17]) or carboxyterminal truncated recombinant purified envelope glycoproteins E1 and E2 (5 μg/mL dissolved in phosphate-buffered saline containing 0.5% betaine)[10,15] in DMEM (Invitrogen, Carlsbad, CA) containing 10% fetal calf serum for 4 hours as previously described.[10,15,17,18] Ligand concentrations used in the assay corresponded to the concentration required for half maximal saturation of ligand-binding to target cells[10,15-17] (Barth et al., unpublished observations, 2006). Procedures for expression and purification of HCV-LPs and insect cell control preparations (GUS) have been described.[10,15-17] HepG2 cells incubated with 3 different, independently prepared control insect cell preparations (for HCV-LPs) or an equal volume of phosphate-buffered saline containing 0.5% betaine (for recombinant envelope glycoproteins) served as negative control for binding experiments.[10,15,17] This approach ensures that the observed changes in gene expression are not induced by insect cell proteins contaminating the HCV-LP preparation or E1/E2 buffer components. Cellular binding of HCV-LPs or recombinant envelope glycoproteins was confirmed via flow cytometry as previously described.[10] Following incubation of HepG2 cells with ligand, total RNA was extracted from 1×10^6 cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany).[19]

Isolation of Human Liver RNA. Tissue samples from liver biopsies from 4 patients with chronic HCV infection or liver resections from 3 patients with liver metastasis from colorectal cancer but without liver disease were snap-frozen in liquid nitrogen, and RNA was isolated as previously described.[19] RNA integrity was confirmed using an Agilent Bioanalyzer and Agilent RNA 6000 Pico assay (Agilent Technologies, Palo Alto, CA) before performing further downstream applications. Total RNA recovered from biopsy material was subjected to a single round of RNA amplification using a commercially available RNA amplification system based on the Eberwine protocol[20] (MessageAmp; Ambion, Austin, TX). Diagnosis of HCV infection was made via detection of HCV RNA using COBAS AMPLICOR HCV v2.0 (Roche Molecular Systems, Pleasanton, CA) and VERSANT HCV-RNA 3.0 Assay (bDNA) (Bayer Corporation Diagnostic, Tarrytown, NY) and anti-HCV antibodies using Anti-HCV-IgG ChLIA (Abbott Laboratories, Abbott Park, IL). Liver biopsy histology of HCV-infected individuals revealed mild inflammatory activity (grade 1-2) and fibrosis (stage 1-2) according to the METAVIR score. Patients did not receive any antiviral treatment before liver biopsy. Approval for this study was obtained from the University Hospital Freiburg institutional review board. Informed consent was provided according to the Declaration of Helsinki.

Microarrays. Complementary DNA (cDNA) microarrays[19] were produced and processed essentially according to the Stanford protocol described by Eisen and Brown.[21] Approximately 7,700 annotated genes from the RZPD (Resource Center and Primary Database, Berlin, Germany) were obtained as bacterial stocks. A list of all the 7,767 genes on the chip is available on the home page of the Core Facility Genomics Web site (www.genomics.uni-freiburg/products/genelist). Sample or reference RNA were transcribed into cDNA in the presence of Cy3- or Cy5-labeled dUTP, respectively.[19] Hybridizations were performed in the presence of an equal amount of reference RNA (Stratagene, La Jolla, CA) as described by Boldrick et al.[22] and in our previous study[19] (www.genomics.uni-freiburg.de). All other steps, including hybridization, were performed following the protocol published by Brown et al. (http://cmgm.stanford.edu/pbrown).

Statistical Analysis. Signal intensities were measured with an Axon 4000A scanner using GenePix 4.1 software (Axon Instruments, Union City, CA). Artifacts were excluded manually if not detected by the software. Image and data files, array layout, and all relevant information according to the MIAME (Minimum Information About a Microarray Experiment[23]) guidelines were transferred to the GeneTrafficDuo database (Microarray Data Management and Analysis Software; Iobion Informatics, La Jolla, CA). To exclude artifacts near background range, all spots were eliminated when sample intensity or reference intensity was less than 50 or less than the local background. Local background was subtracted from spot intensities. Normalization was performed using the locally weighted scatter plot smoother subgrid normalization method.[23] Subgrid normalization calculates the normalization factor for each of the 16 subgrids independently and therefore

Table 1. Primers Used for Semiquantitative RT-PCR and Quantitative Real-Time RT-PCR

Gene Name	GenBank Accession Number	Primer Sequence (5′→3′)	Length (nt)	Position (nt cDNA)	Tm (°C)	Size (bp)
GAPDH	BC004109	F: TGGAAATCCCATCACCATCT	20	210-219	60.13	351
		R: GTCTTCTGGGTGGCAGTGAT	20	541-560	60.12	
Apolipoprotein B100	X04506	F: TGCAGCAGCTTAAGAGACACA	21	6797-6817	59.94	220
		R: GCTCTGAAGGCATTGATTTTC	21	6976-6996	58.92	
IL-1 receptor II	X59770	F: TGAAGGCCAGCAATACAACA	20	639-658	60.26	219
		R: GGGTAGGCGCTCTCTATGTG	20	818-837	59.86	
IgG Fc receptor	T57079	F: AGTTACCAACTCCTGTCTGGTTTC	24	851-874	59.97	266
		R: GTTCCTGACATTTCAGCTCTTCTT	24	1043-1066	60.29	
Ga12 subunit	L01694	F: AGGGCTCAAGGGTTCTTGTT	20	626-645	60.11	218
		R: CAGCTGAAACTCGCTTCTCC	20	805-824	60.28	
Lipocortin	N81077	F: CACCTTCTTCATCAAGCCATGAAAGGTG	28	869-896	61.9	193
		R: CACAAAGAGCCACCAGGATT	20	996-1015	60.1	
PTPase PIR1	AF023917	F: GGACTGGCTACCTCATTTGC	20	474-493	59.70	218
		R: ATTGTGGACTGGTTGCATGA	20	671-690	59.97	
Apolipoprotein E	M12529	F: GGTCGCTTTTGGGATTACCT	20	145-164	60.32	150
		R: TTCCTCCAGTTCCGATTTGT	20	275-294	59.53	
HBP	M64098	F: GGAAGCGACACCGTTGTTAT	20	2086-2105	60.00	694
		R: TCTCCTGGACAACTGGCTCT	20	2741-2760	59.99	
IFNAR2-1	L41944	F: AGGCCTATGTCACCGTCCTA	20	311-330	59.57	218
		R: TCCCTCTGACTGTTCTTCAATG	22	502-523	59.33	
LMP2	Z14977	F: GGAACCTCCACTTGTTTTGG	20	273-292	59.42	242
		R: CTGCACTTCCTCGGGAGAC	19	497-515	59.47	

NOTE. Gene name, GenBank accession number, primer sequences, primer length, position in relationship to the gene cDNA, melting temperature T_m, and PCR product size are shown.

Abbreviations: nt, nucleotide; cDNA, complementary DNA; bp, base pairs; IL-1, interleukin-1; IgG, immunoglobulin G; PTPase PIR1, protein tyrosine phosphatase PIR1; HBP, high-density lipoprotein–binding protein; IFNAR2-1, interferon alpha receptor 2-1; LMP-2, major histocompatibility complex–encoded proteasome subunit 2; F, forward primer; R, reverse primer.

is—compared with global normalization—relatively insensitive to local variations on the array. Applying these criteria, 7,419 genes were subjected to statistical analysis. Following an approach proposed by Dudoit et al.,[24] the computed expression ratios depend on the intensity of the spots. Thus, a smooth nonlinear least squares fit was computed to correct for an intensity-dependent bias. Initially, the log ratio of measured Cy3 and Cy5 values obtained from the image analysis software was computed. A 2-sample *t* test was used for statistical analysis of differentially expressed genes. To adjust the obtained *P* values, the method by Benjamini and Hochberg[25] was applied to control for multiple testing (fdr = false discovery rate). Genes with a *P* value of less than .05 were selected, and agglomerative hierarchical clustering as introduced by Kaufman and Rousseeuw[26] was performed using the R statistical software package (www.r-project.org).

Semiquantitative and Real-Time Reverse-Transcriptase Polymerase Chain Reaction. Procedures for semiquantitative reverse-transcriptase polymerase chain reaction (RT-PCR) have been previously described.[19] PCR products (PCR primers are listed in Table 1) were run on a 3% agarose gel and evaluated in relation to the corresponding GAPDH band using Scion Image software (Scion, Frederick, MD). Real-time PCR was performed on a LightCycler platform (LightCycler Version 3.5; Roche Molecular Biochemicals, Basel, Switzerland) as previously described.[27]

Results

Cellular Binding of HCV-LPs to Hepatoma Cells Modulates Cellular Gene Expression. To characterize the cellular response following binding of HCV envelope glycoproteins to host cells, we incubated HepG2 hepatoma cells with HCV-LPs. Following binding of HCV-LPs to target cells, we performed a genomic analysis of cellular host responses using a 7.5-kd human cDNA microarray.

The microarray analysis of 6 independent experiments entailing more than 45,000 single measurements clearly distinguished the gene expression patterns in HCV-LP–treated cells from the expression patterns of control cells (original data stored according to the MIAME guidelines are accessible at http://www.genomics.uni-freiburg.de [Data download]). A total of 565 out of 7,419 genes (7.6 %) were differentially expressed in HepG2 cells incubated with HCV-LPs compared with control cells (more than 1.5-fold upregulated or downregulated). Transcription of 316 genes was increased more than 1.5-fold, and transcription of 249 genes was decreased more than 1.5-fold in hepatoma cells incubated with HCV-LPs compared

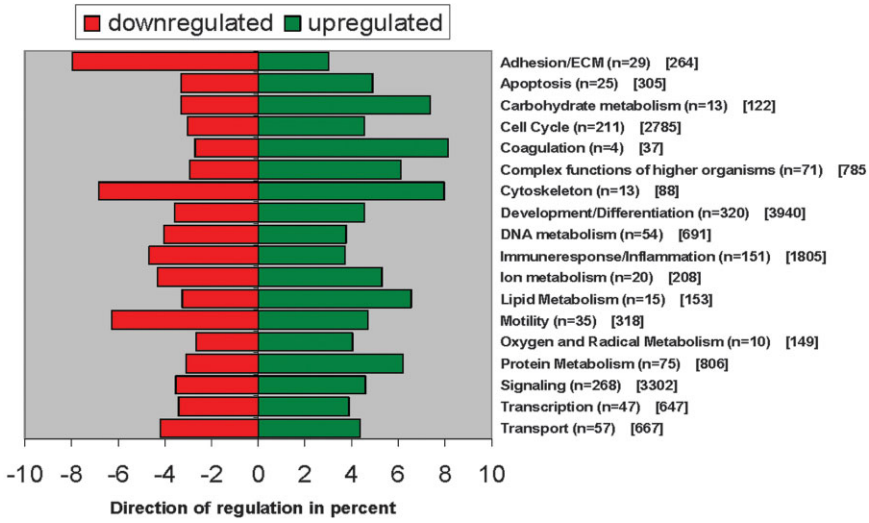

Fig. 1. Functional categories of differentially expressed genes. HepG2 hepatoma cells were incubated with HCV-LP or control preparation as described in Materials and Methods. Four hours after HCV-LP binding, total cellular RNA was extracted and purified, and gene expression profiling was performed using a 7.5-kd human cDNA microarray. Differentially expressed genes (n = 565) in cells binding HCV-LPs versus control cells were annotated and grouped according to functional categories. The graph shows the percentage of differentially regulated genes out of total genes in each category.

with control cells (Supplementary data S1; Supplementary material for this article can be found on the HEPATOLOGY website (http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html). The percentage of genes modulated by HCV envelope–cell surface interaction was in a similar range as the transcriptional changes induced by binding of HIV glycoprotein gp120 to monocyte-derived macrophages.[14]

Examination of transcriptional changes revealed a broad and complex transcriptional program induced by HCV-LP binding and entry to target cells (Fig. 1). Transcriptional changes occurred in all functional categories of genes. Although many of these genes are of unknown function or have never been associated with HCV, our analysis identified at least 19 genes that have been associated with HCV infection, replication, gene expression, or virus–host protein interaction (Table 2). Several of these genes encode for proteins involved in signal transduction, such as ERK1, MKK7b, mitogen-activated protein kinase, or the small GTP-binding protein. Interestingly,

Table 2. HCV-LP–Induced Transcriptional Changes Previously Associated With HCV–Host Interaction

Gene Name	GenBank Accession Number	Cell Line/Tissue	Reference	Confirmation
Apolipoprotein B-100	X04506	Blood (human)	Andre et al.[64]	+
Apolipoprotein E	M12529	Blood (human)	Andre et al.[64]	+
Cartilage GP-39 protein (YKL-40)	Y08374	Liver (human)	Shackel et al.[68]	ND
Chemokine receptor CXCR4	AF005058	Liver (human)	Bieche et al.[66]	ND
Endoglin	X72012	Liver (human)	Asanza et al.[59]	ND
ERK1	AA018162	HepG2 (human)	Giambartolomei et al.[53]	ND
γ-Glutamyltransferase	X98922	Liver (human)	Hoofnagle[58]	ND
Glutathione peroxidase	D00632	Huh-7–derived cell line (human)	Li et al.[56]	ND
High-affinity IgG Fc receptor	T57079	Blood (human)	Maillard et al.[63]	+
Immunoglobulin heavy chain variable region gene	Y14737	B cells (human)	Chan et al.[57]	ND
LMP-2	Z14977	Liver (chimpanzee)	Su et al.,[28] Bigger et al.[42]	+
Macrophage inflammatory protein 1α/RANTES receptor (CCR1)	AI151215	Blood (human)	Lichterfeld et al.[65]	ND
MHC class I HLA-F	AF055066	Liver (chimpanzee)	Su et al.,[28] Bigger et al.[42]	ND
TRAIL	U37518	Huh7 (human)	Lee et al.[55]	ND
MKK7b	AF013589	Huh-7 (human)	Taniguchi et al.[54]	ND
Monocyte chemotactic and activating factor (MCP-1)	M24545	Liver (human)	Nischalke et al.[67]	ND
Monocyte chemotactic protein 2	Y16645	PBMCs (human)	Hellier et al.[61]	ND
Small GTP-binding protein	U18420	Yeast (yeast)	Isoyama et al.[62]	ND
SP1 transcription factor	XM028606	HepG2 (human)	Lee et al.[60]	ND

NOTE. Differentially expressed genes that have been previously reported to be modulated by HCV structural protein expression, replication, or infection are listed along with the associated GenBank accession number, cell line or tissue of observation, and respective reference. Differential expression of randomly selected genes in HCV-LP treated versus control cells was confirmed via semiquantitative (Fig. 2) and real-time RT-PCR (data not shown) in independent experiments using cDNA-specific primers (see Table 1).

Abbreviations: ND, not done; PBMCs, peripheral blood mononuclear cells.

Table 3. Comparative Analysis of Gene Expression Profiling After Binding of HCV-LPs and Recombinant Envelope Glycoproteins E1/E2

Gene Name	GenBank Accession Number	Fold Change	
		E1/E2	HCV-LP
S6 kinase b	AB019245	1.9	1.7
Apolipoprotein E	M12529	1.6	2.5
IgG Fc receptor I	T57079	1.5	2.8
NKG5	M85276	1.5	1.9
Hepatic dihydrodiol dehydrogenase	U05861	1.5	1.9
TSPY-like 2	AB015345	1.5	1.5
Adaptor protein X11beta	AF047348	−1.5	−1.7
Thyroid-stimulating hormone alpha subunit	S70585	−1.5	−1.8
Chemokine ligand 3	M36821	−1.6	−1.5
Caveolin	Z18951	−1.6	−1.7
Rhesus polypeptide (RhII)	X63094	−1.6	−1.8
Erythrocyte plasma membrane glycoprotein	X64594	−1.7	−1.6
Fibronectin receptor alpha subunit	X06256	−1.7	−1.7
Secretory leukoprotease inhibitor (SLPI)	AA460433	−1.7	−2.8
CD44 antigen	AW732334	−1.7	−3.0
Zeta haemoglobin	M24173	−1.8	−1.9
Serin protease with IGF-binding motif	D87258	−1.9	−1.6
D-site binding protein	U48213	−2.2	−1.5
Macrophage inflammatory protein 1α/ RANTES receptor	AI151215	−2.6	−1.8

NOTE. Differences in gene expression from cells incubated with HCV-LPs or insect cell control preparation were compared with gene expression profiles from hepatoma cells incubated with recombinant E1/E2 or buffer (negative control). Genes similarly expressed after HCV-LP and E1/E2 binding are shown.

Abbreviations: HCV-LPs, HCV-like particles; IgG, immunoglobulin G; IGF, insulin-like growth factor.

expression of many of the genes necessary for innate immune responses was modulated by HCV-LP–cell surface interaction (Tables 2, 3; Fig. 2). These genes include major histocompatibility complex class I (HLA-F), major histocompatibility complex class II transactivator, the proteasome subunit LMP-2, the type II interleukin-1 receptor, the high-affinity Fc receptor, the short form of the interferon-α/β receptor chain 2, the monocyte chemotactic protein 1 and 2, macrophage inflammatory protein 1α/RANTES receptor (chemokine receptor [C-C motif] 1), and chemokine receptor (C-X-C motif) 4. Furthermore, similar to recent observations *in vivo*,[28] several genes involved in lipid metabolism were significantly altered following HCV-LP incubation. These genes include apolipoprotein E, apolipoprotein B-100, and high-density lipoprotein–binding protein (Table 2; Fig. 2).

Alteration of HCV-LP–induced gene expression was specific for the interaction of the host cell with HCV structural proteins, because incubation of hepatoma cells with 3 different control insect cell preparations in 3 independent experiments performed in triplicate did not result in modulation of target genes depicted in Supplementary Data S1, Tables 1-4, and Fig. 2 (data not shown).

Hierarchical Clustering Differentiates Hepatoma Cells Incubated With HCV-LPs From Control Cells. To assess whether gene expression between target cells incubated with HCV-LPs was significantly different from target cells incubated with insect cell control preparations, we performed a 2-dimensional hierarchical cluster analysis using 21 genes with statistically significant differences (Supplementary Data S2). The clustering allocated HepG2 incubated with HCV-LPs and control preparation in 2 distinct groups. The dendrogram underlines the

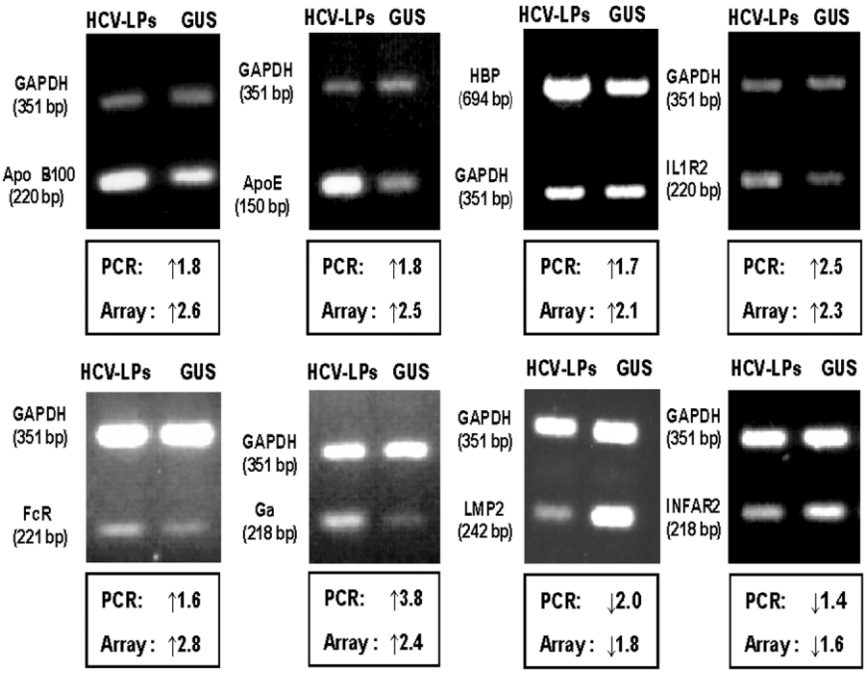

Fig. 2. Confirmation of differential gene expression by semiquantitative RT-PCR of mRNA in HepG2 cells following binding of HCV-LPs or control preparation (GUS). Semiquantitative RT-PCR of transcripts of indicated genes was performed as described in Materials and Methods. Results of a representative independent experiment of the array analysis are shown. Differences in gene expression obtained by microarray analysis and semiquantitative RT-PCR are shown side-by-side. HCV-LPs, HCV-like particles; GUS, insect cell control preparation; PCR, polymerase chain reaction.

Table 4. Similarly and Differentially Regulated Genes Following HCV-LP Binding to Hepatoma Cells and HCV Infection *In Vivo*

Gene Name	GenBank Accession Number	Fold Change	
		In Vivo	In HepG2
Similarly expressed genes			
Radical fringe homolog	U94353	3.9	1.9
Neuropathy target esterase	AJ004832	3.0	1.6
Hepatic nuclear factor 1 (TCF1)	M57732	2.8	1.5
Citrate synthase	AF047042	2.7	3.0
3-Hydroxyanthranilic acid dioxygenase	Z29481	2.7	1.5
KE4 protein	AF117221	1.9	1.7
Myristilated and palmitylated serine-threonine kinase	AF060798	1.9	1.9
IKBL	X77909	1.9	1.8
MHC class II transactivator CIITA	U18288	1.7	1.6
CYP2D7AP pseudogene for cytochrome P450 2D6	X58467	1.7	1.6
Extracellular matrix protein 1	U68187	−1.5	−1.5
MAFA-L (killer cell lectin-like receptor subfamily G, member 1)	AF081675	−1.9	−1.6
Interferon receptor ifnar2-1	AA865870	−2.2	−1.6
GDP-dissociation inhibitor protein (Ly-GDI)	L20688	−2.6	−1.9
Macrophage inflammatory protein 1α/RANTES receptor	L10918	−2.6	−1.8
Osteogenic protein 1	W73473	−2.7	−1.6
Cyclin T1	AF048730	−2.7	−1.5
Vascular endothelial growth factor–related protein VRP	U43142	−3.0	−1.6
Decorin	H11506	−3.1	−1.8
Chemokine receptor CXCR-4	AF005058	−4.0	−1.9
Truncated epidermal growth factor receptor–like protein precursor	U95089	−4.3	−1.5
Caveolin 1	Z18951	−4.6	−1.5
Early growth response protein 1	X52541	−4.8	−1.7
Cartilage GP-39 protein (YKL-40)	Y08374	−5.7	−2.0
Low-density lipoprotein receptor–related protein 1 (LRP1)	R90800	−8.3	−1.7
Monocyte chemotactic and activating factor (MCP-1)	M24545	−18.7	−1.6
Differentially expressed genes			
LMP2	Z14977	5.1	−1.8
Liver dipeptidyl peptidase IV	N24617	4.9	−1.6
Butyrophilin	U97502	3.8	−1.6
n-myc	Y00664	3.8	−1.7
G protein-coupled receptor V28	H17651	3.1	−2.0
Retinoic acid receptor responder 3 (RARRES3)	AF060228	2.9	−1.6
KIAA0352	AB002350	2.8	−1.5
hB-FABP	AJ002962	2.7	−1.6
Prostate associated PAGE-1	AF058989	2.6	−2.3
KIAA0513	AB011085	2.5	−1.5
Telethonin	AJ000491	2.0	−1.9
DT1P1A11	U92992	1.7	−1.6
N-methyl-D-aspartate receptor 2D (NMDAR2D)	U77783	1.7	−1.5
Phenylalanine-tRNA synthetase (FARS1)	AF097441	1.7	−1.6
C2H2 zinc finger protein	AF033199	1.5	−1.6
KIAA0313	AB002311	−9.4	2.0
Prepro-oxytocin-neurophysin I (OXT)	M11186	−7.7	2.1
Carbonic anhydrase I (CAI)	M33987	−6.5	2.5
Zinc finger protein (MBLL)	AF061261	−5.9	1.8
Dead box X isoform (DBX)	AF000982	−5.5	2.0
RNA-binding protein Gry-rbp (GRY-RBP)	AF037448	−5.4	1.6
General transcription factor 2-I (GTF2I)	AF035737	−4.5	2.1
Smooth muscle protein (SM22)	M95787	−3.6	1.8
Aorta caldesmon	M83216	−3.3	1.6
TNF-related apoptosis–inducing ligand (TRAIL)	U37518	−3.1	2.0
Apolipoprotein B-100	X04506	−2.4	2.6
Neutral amino acid transporter B	U53347	−2.3	1.7
Clone zap113	L40400	−2.3	1.7
Cullin 1	AF062536	−2.2	1.6
Carbonic anhydrase precursor (CA 12)	AF037335	−2.0	1.8
Osteogenic protein	X51801	−2.0	2.0
Synaptotagmin	M55047	−1.7	1.9

NOTE. Genomic analysis of cellular host responses in liver tissue samples from 4 HCV-infected individuals versus 3 non–HCV-infected controls was performed as described in Materials and Methods. Differences in gene expression from liver tissue samples from HCV-infected individuals versus non–HCV-infected controls were compared with gene expression profiling from HCV-LP treated versus control cells. Genes similarly and differentially expressed during HCV infection *in vivo* and following HCV-LP binding *in vitro* are shown.

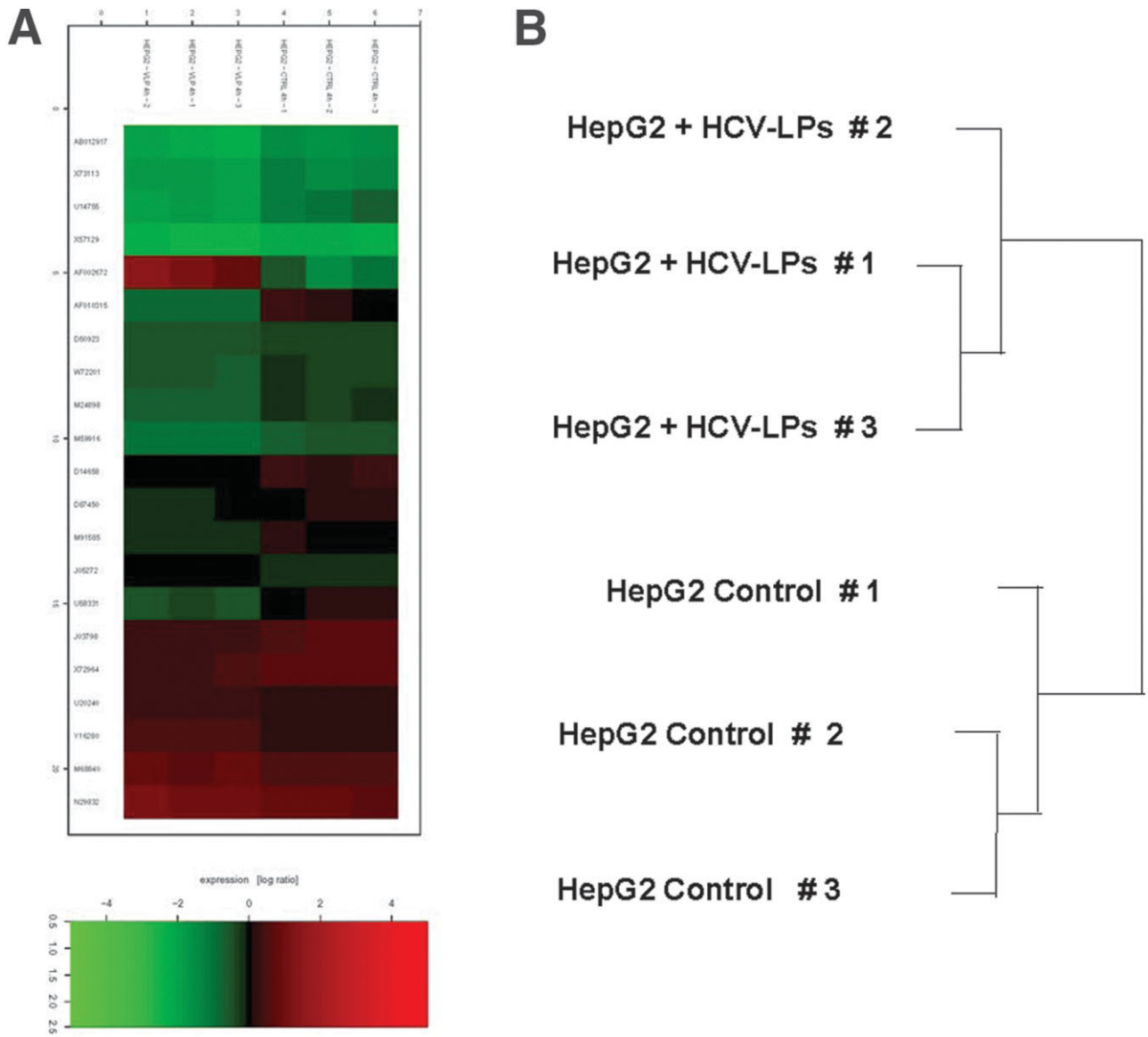

Fig. 3. Hierarchical cluster analysis of gene expression. (A) Hybridization results of 21 genes (shown in Supplementary Data 1) were clustered using the R statistical software package. Changes in gene expression in HCV-LP–incubated HepG2 cells and HepG2 cells incubated with insect cell control preparation versus reference RNA are indicated by a color scale (red, upregulation of transcription; green, downregulation of transcription). (B) Tree depicting the correlation in cellular gene expression of different experiments. HCV-LPs, HCV-like particles.

close relationship of expression profiles in HCV-LP–incubated versus control-incubated host cells (Fig. 3).

To specifically examine the events induced by cellular binding of viral envelope glycoproteins present on the surface of HCV-LPs, we incubated hepatoma cells with highly purified recombinant envelope glycoproteins E1 and E2 in side-by-side experiments (Supplementary Data S3). Interestingly, our analysis identified 19 host cell genes similarly regulated followed incubation of HCV-LPs and recombinant E1 and E2 with target cells (Table 3). These results suggest that HCV initiates the induction of host cell responses following the very first contact of viral envelope glycoproteins with host cell surface molecules.

Interestingly, the observed changes were completely different from virus-induced changes observed in HepG2 cells transfected with replication-competent hepatitis B virus cDNA,[29] suggesting that the host responses induced by HCV-LPs and recombinant envelope glycoproteins are specific for HCV–cell interaction and do not represent a nonspecific cellular response induced by heterologous viral proteins.

Verification of Differentially Expressed Genes via RT-PCR. Ten genes were randomly selected. Their dif-

ferential expression was confirmed via semiquantitative RT-PCR and/or quantitative real-time RT-PCR analysis of total cellular RNA (Tables 1, 2; Fig. 2). For verification of transcriptional changes, we performed an additional independent series of binding experiments. Although semiquantitative RT-PCR may underestimate differences in gene expression, the results obtained via RT-PCR closely mirror the microarray data (Fig. 2). Quantitative real-time RT-PCR confirmed transcriptional changes identified by microarray analysis, including expression profiles of 2 additional genes (lipocortin and protein tyrosine phosphatase PIR1, data not shown). These data indicate that microarray analysis represents a valid and reproducible method for the detection of qualitative transcriptional alterations induced by envelope–target cell interaction.

Impact of Differential Gene Expression Induced by Cellular HCV Binding In Vivo. To assess the impact of these findings for HCV infection in vivo, transcriptional changes induced by cellular HCV-LP binding were compared with gene expression profiles in liver tissue samples from HCV-infected individuals. Thus, we performed a genomic analysis of cellular host responses in liver tissue samples from 4 HCV-infected individuals versus 3 non–HCV-infected controls using the same 7.5-kd human cDNA microarray. A 2-dimensional hierarchical cluster analysis distinguished the gene expression profile of HCV-infected liver versus control liver in 2 distinct groups (Supplementary Data S4). A total of 703 out of 7,419 genes (9.5%) were found to be significantly regulated in the HCV-infected liver versus control livers (upregulated or downregulated more than 1.5-fold, $p < 0.05$ for the false discovery rate; Supplementary Data S5). Side-by-side analysis revealed that the expression of 26 genes was similarly altered following HCV-LP binding in hepatoma cells and viral infection *in vivo* (Table 4). Several of the proteins encoded by these genes are involved in cell signaling and regulation of transcription, such as hepatic nuclear factor 1 (TCF1), IKBL, major histocompatibility complex class II transactivator, and cyclin T1. Moreover, HCV-LP binding to hepatoma cells and HCV infection *in vivo* both modulated the expression of genes involved in immune responses. These genes encode members of the chemokine family of proteins, including chemokine receptor (C-C motif) 1, chemokine receptor (C-X-C motif) 4, monocyte chemotactic protein 1, and MAFA-L (killer cell lectin-like receptor subfamily G, member 1).

On the other hand, the expression of several genes was different between responses induced by HCV-LP binding and during HCV infection *in vivo* (Table 4). The modulation of these genes is most likely the result of virus–host interactions requiring ongoing productive viral infection. Antiviral immune responses within the liver not present in the *in vitro* systems may also contribute to the differential regulation of genes observed *in vitro* and *in vivo*.

Discussion

Using recombinant envelope glycoproteins and noninfectious HCV-LPs as a model ligand for HCV particle–host cell surface interaction, we demonstrate that binding of HCV to host cells results in a marked modulation of gene expression. Recombinant envelope glycoproteins and HCV-LPs have been shown to bind to target cells in a receptor-mediated manner; HCV-LPs have been shown to enter human hepatocytes, hepatoma cells, and dendritic cells, thus providing a convenient model for the study of envelope–host cell interactions.[9,10,15,17,18,30,31] In contrast to retroviral HCV pseudotypes,[32,33] HCV-LPs do not contain heterologous retroviral proteins, thus allowing assessment of HCV-specific changes following envelope HCV–host interaction. HepG2 cells have been shown to bind specifically recombinant E2, HCV-LPs, and HCV virions[10,15,17,30,31,34-36] and have been used as a hepatocyte model cell line to study HCV–host interaction and pathogenesis of HCV infection.[37-39] Although infection of tissue culture–derived HCV has thus far been demonstrated only in Huh-7 hepatoma cells,[2-4] HepG2 cells have been shown to be susceptible to entry of HCV-LPs,[10] serum-derived virions,[7,40] and replication of defined replicons.[41] Confirmation of transcriptional changes induced by HCV-LP binding during HCV infection *in vivo* (Table 4) underlines the relevance of the used model system for HCV infection *in vivo*. Further studies using other hepatoma lines (Huh7.5, Hep3B) are underway to study the impact of cell line and cell surface receptor–specific factors for host cell responses.

In line with recent observations for other viruses,[11] our results indicate that HCV–host cell membrane interaction is not limited to the cues given to the virus by the cell resulting in cellular binding and entry of the virus, but results in a cascade of signals altering the expression profile of the host cell. Because HCV-LPs are not able to replicate, our data suggest that part of the antiviral host responses observed *in vivo* does not require viral replication but is attributable to the signals induced by the very early steps of virus–host interaction. To distinguish signals specifically induced by binding of viral envelope glycoproteins to the host cell surface from signals induced during internalization and entry, we compared modulation of gene expression following incubation of hepatoma cells with recombinant envelope proteins and HCV-LPs (Supplementary Data S3; Table 3). Our data indicate that several of the events induced during HCV-LP internaliza-

tion are mediated by binding of the envelope glycoproteins to target cells (Table 3). On the other hand, the overall expression profile induced by the 2 ligands showed marked differences (Supplementary Data S2 and S3), suggesting that each step during viral binding and internalization results in a specific pattern of host cell responses. Differences in protein conformation or the presence of a functional core protein within HCV-LPs may also contribute to these differences.

The functional relevance and biological significance of the observed alteration of host cell expression was demonstrated by the side-by-side analysis of findings obtained in the *in vitro* model systems with host cell responses induced by HCV infection in the human liver (Table 4). Our results demonstrate that differential expression of several host cell genes induced by HCV-LP binding (Table 2; Fig. 2) was also detected in genomic analyses of HCV-infected liver tissue *in vivo* in HCV-infected humans (Table 4). Furthermore, differential expression of several host genes identified in this study has also been observed during the very early phase of acute HCV infection in chimpanzees.[28,42] Taken together, the confirmation of changes in modulation of gene expression *in vivo* clearly demonstrates the biological significance of the events identified in this study.

First, we found a modulation of innate immune responses. Host responses included the upregulation of type II interleukin-1 receptor and high-affinity immunoglobulin G Fc receptor. Because type II interleukin-1 receptor plays an important role in mediating innate antiviral immune responses,[43] HCV-induced upregulation of type II interleukin-1 receptor expression may interfere with type II interleukin-1 receptor signaling[44] and counteract innate antiviral defense strategies. Upregulation of the $F_c\gamma$ receptor has been previously shown to be a strategy for HIV and other viruses to facilitate entry of virion–antibody complexes.[45] Furthermore, HCV-LP binding and internalization resulted in a downregulation of the proteasome subunit LMP-2 (Table 2, Fig. 2). Proteasome-mediated degradation of viral antigens represents a key in the cascade of proteolytic processing required for the generation of peptides presented at the cell surface to cytotoxic T lymphocytes by major histocompatibility complex class I molecules.[46] Although the lack of model systems for the study of HCV antigen presentation in hepatocyte-derived cell lines does not yet easily allow study of the functional relevance of this observation for HCV antigen presentation, the analysis of antigen presentation of other viruses suggests that modulation of LMP-2 expression may play a role in HCV pathogenesis. A well-characterized example is adenovirus 12 suppressing specifically the expression of LMP-2 and LMP-7 genes, allowing adenovirus transformed cells to escape immune surveillance.[47] A recent study also suggested an important role of proteasomal processing in the escape of HCV.[48] Furthermore, an alteration of immunoproteasome subunit gene expression has been associated with HCV clearance in chimpanzees: whereas chimpanzees with subsequent viral clearance exhibited a rapid and strong increase of LMP-2 expression during the first weeks of viral infection, chimpanzees with persistent infection were characterized by a flat curve with blunted response of LMP-2 expression.[28] This finding suggests that the observed downregulation of LMP-2 expression may contribute to the interplay of virus and host cell responses during the early phase of acute viral infection.

Other changes induced by HCV-LP binding and detected in the liver of human HCV-infected individuals included the downregulation of defined chemokine receptors, including chemokine receptor 1 and chemokine receptor 4 (Table 4). Modulation of chemokine receptor expression may allow the virus to counteract innate antiviral defense strategies as previously shown for HIV,[49] human cytomegalovirus,[50] and human herpesvirus 6 and 7.[51]

Furthermore, we observed a modulation of transcription of several genes associated with fatty acid biosynthesis and lipid metabolism (Tables 2-4; Fig. 2). HCV is known to cause the formation of hepatocellular lipid droplets where HCV proteins[52] have been shown to localize. Molecules that block fatty acid biosynthesis have been shown to inhibit HCV replication, suggesting that alteration of fatty acid biosynthesis and lipid metabolism observed during HCV infection may facilitate viral replication.[28] Interestingly, an alteration of genes involved in lipid metabolism has also been observed during the acute phase of HCV infection in chimpanzees supporting the biological significance of the identified events *in vivo*.[28]

In conclusion, for several viruses (including HIV and herpes viruses), it has been demonstrated that the virus takes advantage of the cell's own signal transduction systems to transmit signals to the cells.[11-14] These signals induce changes that may facilitate entry, prepare the cells for invasion, and neutralize host defenses.[11,14] Thus, it is conceivable that the transcriptional reprogramming of liver cells during virus binding and internalization observed in this study may be part of HCV strategy to facilitate viral infection and escape from innate host cell responses. Further analysis of altered gene expression induced by HCV–host cell membrane interaction may provide new insight into the mechanisms underlying viral immune escape and persistence.

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