

The differences in gene expression profile induced by genotype 1b hepatitis C virus core isolated from liver tumor and adjacent non-tumoral tissue

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ABSTRACT

Background: Core protein of the hepatitis C virus (HCV) has an important role in HCV self-replication, pathogenesis and carcinogenesis.

Objectives: To identify the effect of core proteins from different quasispecies of HCV genotype 1b expressed in a HepG2 cell line on human gene expression profiles.

Materials and Methods: Core protein eukrocytic expression plasmids (pEGFP-N1) containing different quasispecies core protein genes of genotypes 1b HCV derived from of HCV-related hepatocellular carcinoma (HCC) tumoral tissue (T) and non-tumoral tissue (NT) were constructed and then transfected to HepG2 cell line. The gene expression spectrum in the cell expression core proteins from T and NT were compared with those in the control by Affymetrix human genome HG-U 133 plus 2.0 microarray.

Results: Different gene expression profiles were acquired between HepG2 expressing core proteins derived from T and NT tissues. Both core proteins caused the modulation of several genes that are up/down-regulated as compared to control, including genes involved in oncogenesis, signal transduction, cell apoptosis, and cell growth cycle regulation. Surprisingly, only one gene—CSNK1A1—was up-regulated by both T and NT core variants.

Conclusions: Core proteins isolated from tumoral or non-tumoral nodules mediate expression of different cellular genes suggesting that variants isolated from different quasispecies may have different biological effects.

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► Implication for health policy/practice/research/medical education:

The study of the role of core protein of HCV is recommended for all basic scientists especially those involved in treatment of HCC.

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Background

Chronic infection with hepatitis C virus (HCV) is asso-

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ciated with the development of liver cirrhosis and hepatocellular carcinoma (HCC). Approximately 3% of the world population is persistently infected with HCV. Since HCV is a serious threat to human health, it is necessary to develop protective and therapeutic measures for controlling the HCV infection. HCV is an enveloped, positive-sense; single-stranded RNA virus of 9600 nucleotides

in length. It encodes a polyprotein precursor of almost 3000 amino acids. The polyprotein is post-translationally processed by cellular and viral proteases into the mature structural (core, E1, E2) and nonstructural proteins (1). HCV core protein consists of 191 amino acids, which is a central component of the virion and necessary for nucleocapsid formation. A series of research studies have previously reported that the core protein is involved in regulation of cellular transcription, virus-induced transformation, and pathogenesis of HCC. It is also implicated in the development of HCC in addition to its function as a structural protein (2). The core protein can also induce hepatocellular mitochondrial injury, oxidative stress, and antioxidant gene expression (3). The constitutive expression of HCV core protein can induce HCC in transgenic mice; the expression level of HCV core protein in the liver in these mice was similar to that in patients with chronic hepatitis C (4). Therefore, some investigations have been focused on HCV core protein which revealed that HCV core protein may contribute to mammalian cells growth regulation (5). However, detailed molecular mechanisms are not still clear. The HCV genome exhibits a considerable degree of variation and is currently classified into six genotypes and more than 60 subtypes (6, 7). Even in the same subtype, there exist quasispecies. Quasispecies constitute a pool of viral variants that can change and acquire new selective advantages in a very short time. These new variants have adaptive advantages with a modified viral tropism, host range, virulence and drug resistance. This genetic variability may have several important effects with respect to the promotion of viral persistence and pathogenesis, and it may generate viral escape mutants from the immune system and resistance to interferon therapy. Indeed, a switch from acute to chronic infection has been associated with a wider variety of quasispecies (8). Another property of these quasispecies is their compartmentalization into different cell types such as the liver or peripheral blood mononuclear cells (9). Therefore, patients infected with different HCV genotypes, even with different quasispecies of the same genotype, might have different clinical features (10) and their own characteristics pathogenesis, which might be caused by different functions of core protein derived from different quasispecies. Recent studies suggest that gene expression profiles of various genotypes 1b, 2a, and 4d of HCV core proteins were different in the Huh-7 cell line with microarray analysis (11). More interestingly, our previous studies indicated that core proteins isolated from HCV-associated HCC tumoral tissue (T) and non-tumoral tissue (NT) have some differences in their pathogenesis in HCV persistent infection and HCC (12). Indeed, the interferon-inducible protein kinase R (PKR) can be activated and co-localized by core protein from T but not from NT (12). HCV core protein variants isolated from T but not from NT interact with Smad3 and inhibit the TGF- β pathway (13). Proapoptotic and pronecrotic effect of different truncated HCV core protein derived from T and NT inhibit the growth of Chang liver cells and

HepG2 cells at different levels (14, 15). However, detailed molecular mechanisms explaining these effects are little known. With the recent development in DNA microarray technology, a type of high-throughput analysis for gene expression has opened a new era in medical sciences. Analysis by microarray can determine the patterns of differential gene expression or compare the differences in mRNA expression levels between different cells.

Objectives

To further understand the biological function of HCV core proteins isolated from different quasispecies of genotype 1b and their pathogenic mechanism, we investigated the effects of core proteins derived from T and NT on human gene expression profile by microarray analysis.

Materials and Methods

Core gene amplification and plasmid construction

Two HCV core nucleotide sequences from T (tumoral tissue taken from one patient with HCV-related HCC) and NT (non-tumoral tissue from the same patient) were amplified by polymerase chain reaction (PCR). Primers were designed according to core protein genes:

T: 1-172 aa: 5' CGCGCTAGCATGAGGCACGAATCC 3' (1-14, up), 5' CCGGAATTCGCAACCGGGCAG 3' (505-516, down),
NT: 5' CGCGCTAGCATGAGCAGCAATCC 3' (1-14, up),
5' GAATTCGGCAACCGGGCAGATTC 3' (505-516, down)

PCR products were purified and cleaved with restriction enzymes Nhe I and EcoR I, then re-purified, and cloned into Nhe I and EcoR I-digested and purified pEGFP-N1 to yield Core-GFP fusion plasmids.

Cell culture

The human hepatoma cell line HepG-2 was grown at 11.5(DMEM, Invitrogen, France) supplemented with 10% fetal bovine serum (FBS, Invitrogen, France), 2 mM L-glutamine, 100 U/mL penicillin, 100 μ g/mL streptomycin and 2.5 μ g/mL plasmocin. About 2×10^6 cells were seeded in 10 cm dishes; transfection was performed 24 h after plating with Lipofectamine™ 2000 reagent (Invitrogen) following the manufacturer's instructions. Two μ g of either blank vector pEGFP-N1 (P), pEGFP-N1 (NT) or pEGFP-N1 (T) were used to achieve transfection. After 6 h, the medium was changed and cells were collected after another 24 h post-transfection.

RNA isolation, synthesis of double-stranded cDNA and biotin-labeled cRNA transcripts in vitro

Total cellular RNA was extracted with the Rneasy Mini kit (Qiagen, CA) according to the manufacturer's instructions. The total RNA was evaluated with a spectrophotometric analysis at 260 nm and 280 nm to determine RNA concentration and purity, respectively. The cDNA was synthesized with the reverse SuperScript Choice System (Invitrogen, CA). cRNA was synthesized from approxi-

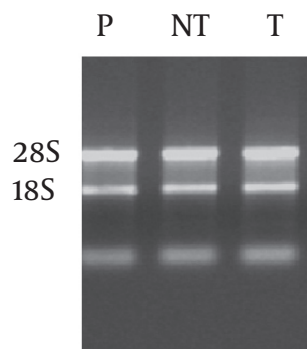


Figure 1. Analysis of RNA extracted from GFP, GFP-core protein (T) and GFP-core protein (NT) transfected cells: Total RNA was extracted from cells transfected with blank pEGFP-N1 (P), pEGFP-N1-core protein (T) and pEGFP-N1-core protein (NT) and analyzed by agarose gel electrophoresis.



Figure 3. Western blot analysis of core protein expression: proteins were extracted from cells transfected with blank pEGFP-N1 (P), pEGFP-N1-core protein (T) and pEGFP-N1-core protein (NT) and Core protein expression was estimated by Western blot using an anti-core antibody.

mately 1 µg double-stranded cDNA template using the RNA Transcript Labeling kit (Enzo Life Sciences, NY). The cRNA was fragmented in a buffer containing 2× MES, 1.7 M NaCl, 40 mM EDTA and 0.02% Tween 20.

Eukaryotic target hybridization and gene expression analysis by microarray

Biotinylated cRNA probes (approximately 15 µg) were hybridized to an Affymetrix human genome HG-U 133 Plus 2.0 array, respectively for 16 h at 45 °C in Affymetrix Gene Chip® hybridization oven 640. The array were stained with stain buffer 1 (2× MES stain buffer, 50 mg/mL acetylated BSA [Invitrogen], 1 mg/mL streptavidine-phycoerythrin [Molecular Probes]) and stain buffer 2 (2× MES stain buffer, 50 mg/mL acetylated BSA, 10 mg/mL normal goat IgG, 0.5 mg/mL biotinylated antibody [Sigma]) and washed with buffer A (6× SSPE, 0.01% Tween 20, 0.005% antifoam) and buffer B (100 mM MES, 0.1 M NaCl, 0.01% Tween 20) in Affymetrix Gene Chip® Fluidics Station 450. The fluorescent intensities of arrays were measured with Affymetrix GeneArray laser confocal scanner (Hewlett Packard, USA).

SDS-PAGE and Western blotting

A part of the cells collected for microarray, was lysed in 300 µL cell lysis buffer (20 mM Tris pH 7.8, 150 mM NaCl, 0.5 mM EDTA, 0.5 mM PMSF, NP40 0.2%) and frozen at 80 °C for 15 min. Homogenates were centrifuged at 12,000

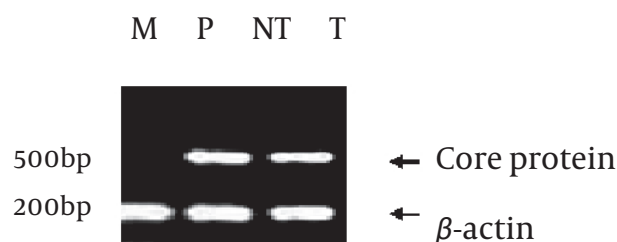


Figure 2. Analysis of core mRNA expression: Total RNA was extracted from cells transfected with blank pEGFP-N1 (P), pEGFP-N1-core protein (T) and pEGFP-N1-core protein (NT) and PCR was performed using primers specific for the HCV core protein.

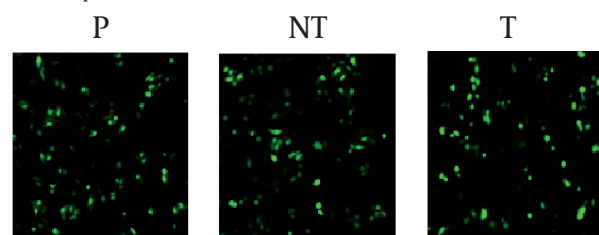


Figure 4. Expression of GFP, GFP-core protein (T) and GFP-core protein (NT) ×20 GFP expression of HepG2 cells transfected with blanks pEGFP-N1 (P), pEGFP-N1-core protein (T) and pEGFP-N1-core protein (NT) was estimated by fluorescence microscopy.

rpm for 5 min. Proteins were quantified with a Beckman 600 using the Bio-Rad protein assay (Bio-Rad, France). Fifty micrograms of total proteins were separated onto a SDS 10% polyacrylamide gel electrophoresis, transferred onto a 0.22 µm nitrocellulose membrane. The membrane was first blocked with the blocking solution for 30 min, then washed twice with water, incubated with the primary antibodies for 1 h, washed for three times, and then blotted with the second antibody. Proteins were visualized using a chemiluminescence detection kit (ECL, Amersham; Pharmacia, USA).

Flow cytometric analysis of GFP

When cells were collected for microarray analysis, the percentage of cells expressing different pEGFP-N1, pEGFP-N1 (T) and pEGFP-N1 (NT) was estimated by FACS analysis, the positive FL1 gated cells representing the GFP expressing cells.

Table 1. Down-regulated genes in HepG2 cells transfected with HCV 1b pEGFP-N1 (T) compared with a blank plasmid (Two genes with unknown function)

Gene names	Fold change (log 2)	GeneBank (No.)
TncRNtrophoblast-derived non-coding RNA	-1.1	AV699657
BIRC4baculoviralIAPrepeat-containing4	-1.4	BE380045

Table 2. Up-regulated genes in HepG2 cells transfected with HCV 1b pEGFP-N1 (T) compared with a blank plasmid (12 genes with unknown function)

Gene names	Description	Fold change (log 2)	GeneBank (No.)
MBNL2	Muscleblind-like 2	1.3	NM018615
LOC126731	LOC126731	1.3	NM145257
FLJ25811	FLJ25811 protein	1.9	AK098766
SPINK5L3	Serine PI Kazal type 5-like 3AK054753	1	AK054753
DKFZp686L1818	hypothetical protein	1.8	AL833167
APOB	apolipoprotein B	3.3	NM000384
ERBB4	erythroblastic leukemia viral oncogene homolog 4	1.8	NM005235
SIRPB1	signal-regulatory protein beta 1	1	NM006065
CNTN5	contactin5	2.3	NM014361
GRIA3	glutamate receptor, ionotropic, AMPA 3	2	NM000828
HIST1H2BG	histone 1, H2bg	1.1	BC001131
C16orf34	chromosome 16 open reading frame 34	1	T53900
CYBB	cytochrome b-245, beta polypeptide	1.7	S67289
BCMO1	beta-carotene 15,15'-monooxygenase 1	3.3	NM017429
CHD9	chromodomain helicase DNA binding protein 9	3.1	NM025134
TBC1D14	TBC1 domain family, member 14	1.4	AI743207
C10orf75	Chromosome 10 open reading frame 75	1.9	AU151788
DKFZp434H222	Hypothetical protein DKFZp434H2226	1.5	AL137370
CSNK1A1	Casein kinase 1, alpha 1	1.5	AI674461
FLJ32642	Hepatocellular carcinoma related protein 1	1.7	D53659

Data analysis

Data were analyzed using the Affymetrix Microarray Suite 5.0 software to filter out genes that were called present in the transfected cells and control cells. We included in our list only those genes that met the quality-control criteria. In data analysis, a threshold was set to eliminate all genes that were not increased or decreased by 2-fold change in a comparison between the transfected cells and control cells

Results

Assay of RNA quality

The amounts and purity of total RNA were evaluated with a spectrophotometric analysis at 260 and 280 nm, respectively. RNA electrophoresis also showed that the 18S and 28S were not degraded, and that highly purified total RNA was acquired, suitable for the requirement of microarray analysis (Figure 1).

Assay of core RNA and protein expression

Using RT-PCR, we analyzed the NT and T core mRNA expression, from RNA extracted from transfected cells (Figure 2). Using Western blot analysis, core protein expression was found in comparable amounts in cells transfected with either plasmid containing T or NT core cDNA (lanes T and NT) but not in lane P (protein from

cells transfected with blank plasmid) (Figure 3). The efficiency of transfection expressed as the percentage of GFP expressing cells was estimated by fluorescent microscope observation to be about 40%-50% (Figure 4).

Identification of genes differentially expressed in HepG2 cells

In present experiments, commercially Affymetrix human gene chip, the HG-U133 plus 2.0 array set which covers the entire human genome was used. This microarray was used to study gene expression profiles of transfected HepG2 cells expressing the HCV genotype 1b core proteins in comparison with cells expressing the GFP protein alone. The gene expressions obtained were analyzed to identify genes up- or down-regulated in each transfected population according to statistical criteria described in the methods section. We applied two-fold changes for a comparison of gene expression profiles induced by the two quasispecies of HCV genotype 1b core protein. The data showed that there were few up/down-regulated genes that meet this criterion (Table 1-5). Compared with the blank vector, 36 genes were found to be modulated by T core expression (32 up-regulated containing 12 genes with unknown function, and four down-regulated containing two genes with unknown function) and 43 were found to be modulated by NT core expression, all of which were up-regulated.

Table 3. Up-regulated genes in HepG2 cells transfected with HCV 1b pEGFP-N1 (NT) compared with a blank plasmid (18 genes with unknown function)

Gene names	Discription	Fold change (log 2)	GeneBank (No.)
ADAMTS17	A disintegrin-like and metalloprotease	2	NM139057
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ZNF277	zinc finger protein (C2H2 type) 277	1.3	BC020626
MCF2	MCF2 cell line derived transforming sequence	1.3	AB085901
IGSF4D	immunoglobulin superfamily, member 4D	1.9	AF538973
CSNK1A1	Casein kinase 1, alpha 1	1	BQ025347
RAB9P40	Casein kinase 1, alpha 1	3.7	AL832249
LOC144871	hypothetical protein LOC144871	1	BG913589
DNAPTP6	DNA polymerase-transactivated protein 6	1.4	AF147415
PINX1	PIN2-interacting protein 1	3.9	BC020930
LOC162993	hypothetical protein LOC162993	1.6	BC037359
CDC14A	CDC14 cell division cycle 14 homolog A	1.1	NM003672
MRAS	muscle RAS oncogene homolog	1.4	NM012219
RPGR	retinitis pigmentosaGTPase regulator	1	NM000328
HIST1H2B	histone 1, H2bg	1.1	BC001131
CYBB	cytochrome b-245, beta polypeptide	1.1	S67289
FLJ12505	hypothetical protein FLJ12505	1.4	NM024749
WINS1	WINS1 protein	2.4	NM018148
TMEM27	transmembrane protein 27	1.3	AF229179
GDEP	gene differentially expressed in prostate	3	AA226269
C6orf209	Chromosome 6 open reading frame 209	1.4	AU146081
LOC23117	Phosphatidyl inositol 3-kinase-related protein kinase	1	BE972419
DKFZp762A217	hypothetical protein DKFZp762A217	1.8	AI765006
ATP11B	ATPase, Class VI, type 11B	1.3	AA639797

Comparison of gene profiles induced by core proteins from T and NT

In comparing T and NT core protein expression, there were 24 different gene expressions—21 up-regulated containing 11 genes with unknown function, and three down-regulated containing one gene with unknown function. CSNK1A1 was up-regulated both in T and NT. RAB9P40

and PINX1 were obviously up-regulated in NT; APOB and FLJ32642 were obviously up-regulated in T; CSNK1A1 and PINX1 were obviously up-regulated in NT compared with T. Regarding the current microarray analysis, the gene expression profiles were mostly involved the oncogene FLJ32642; signal transduction genes CSNK1A1, GRIA3, MRAS; apoptosis related gene BIRC4; cell growth cycle regulation genes PINX1, ERBB4, MCF2, CDC14A; the genes

Table 4. Up-regulated genes in HepG2 cells transfected with HCV 1b pEGFP-N1 (NT) compared with HCV 1b pEGFP-N1 (T) (11 genes with unknown function)

Gene names	Discription	Fold change (log 2)	GeneBank (No.)
MGC2046	hypothetical protein MGC20460	1.9	BC011923
CSNK1A1	Casein kinase 1, alpha	1	BQ025347
PINX1	PIN2-interacting protein	2.2	BC020930
TncRNA	trophoblast-derived noncoding RNA	1.2	AU155361
MGC2752	hypothetical protein MGC2752	1.3	AI651726
CLCN5	Hypothetical protein LOC158563	2.9	AK021494
DEFB126	defensin, beta 126	2.9	AL360078
TncRNA	trophoblast-derived non-coding RNA	1.3	AV699657
C3orf6	chromosome 3 open reading frame 6	1.3	BF696931
ZDHHC21	zinc finger, DHHC domain containing 21	2.3	AA564788

Table 5. Down-regulated genes in HepG2 cells transfected with HCV 1b pEGFP-N1 (NT) compared with HCV 1b pEGFP-N1(T) (One gene with unknown function)

Gene names	Description	Fold change (log 2)	GeneBank (No.)
MBD2	methyl-CpG binding domain protein 2	-3	NM003927.2
ISYNA1	myo-inositol 1-phosphate synthase A1	-1.1	BF976372

participating in ion channel regulation: CLCN5, GRIA3, RAB9P40, RPGR, ATP11B; the gene participating in the process of host resistance to infection and regulating inflammatory reaction: DEFB126, CYBB; transcription regulation genes ZBTB11, 2 MBD2, ZNF277; and other genes of unknown function (Table 1-5).

Discussion

The core protein has been suggested to exhibit various biological functions to the host cell, which might affect the host cells at various stages during the course of cellular malignant transformation and be substantially involved in the pathogenesis of HCV-related diseases (1, 2, 16, 17). Its exact mechanism in HCV persistence infection and HCC, however, still remains unclear (18-20). cDNA microarray analysis can obtain expression information of tens of thousands of genes in the level of RNA at the same time and has been generally applicable to gene expression spectrum research (21, 22), which can not only reveal the mechanism of HCV persistent infection and carcinogenesis, but also provide renewal reference for the clinical diagnosis and treatment of HCV infection. In the present study, we investigated the changes in the gene expression of HepG2 expressing genotype 1b HCV core proteins isolated from T and NT nodules of the same patient using a commercially available Affimetrix U133 Plus 2.0 array containing more than 40,000 genes of human, produced recently by Affimetrix Company (23, 24). The gene expression spectrum regulated by T and NT of genotype 1b core proteins is mainly involved in cell signal transduction, cell apoptosis; cell growth cycle regulation; ion channel regulation; host resistance to infection and regulation of inflammatory reaction; transcription regulation; unknown function, etc. Some pathogenesis/oncogenesis gene expressions are up/down-regulated simultaneously in the HepG2 cell line. Differences in gene expression profiles between T and NT were also found in our former study (15). The findings suggest that HCV CORE could induce apoptosis and necrosis, and different truncated COREs could induce cell apoptosis and necrosis at different levels. Among the same length CORE derived from T, NT and C191 samples, the cell apoptosis and necrosis percentage of T was the highest, and C191 was the lowest (T > NT > C191). HCV CORE could induce apoptosis and necrosis of cells, which might play an important role

in the pathogenesis of HCV persistent infection and HCC; different CORE domains of different HCV quasiespecies might have some difference in their pathogenesis too. From preliminary results of microarray analysis, there were gene expressions characteristics for each core protein. It is indicated that compared with blank vector, the expression of HCV 1b core protein derived from T sample on anti-apoptosis gene was down-regulated, on DKFZ-p686L1818 on dissolving protein and peptide was up-regulated, on gene APOB participating in lipid metabolism and signal transduction was up-regulated (25, 26), and on genes ERBB4, SIRPB1 and CYBB participating in cell multiplication and transmembrane receptor protein signal transduction was obviously up-regulated. Compared with blank vector, core protein derived from NT sample on the following genes were up-regulated: ADAMTS17 dissolving protein and peptide; transcription regulation gene ZNF277, ZBTB11; cell growth regulation gene MCF2 and signal transduction gene CSNK1A1, MRAS; cell growth negative regulation gene PINX1; cell multiplication gene CDC14A and the humeral immunity gene CYBB resisting causative organism infection. It has been suggested that gene expression profiling of HCC and non-tumoral liver tissue may improve prediction of recurrence-free survival (RFS), aid in understanding of the underlying liver disease, and guide patient management. Gene expression profile was also used to determine the molecular signature of HCV-associated HCC and to develop a predictor of RFS. Tsuchiya found that microarray analysis of the non-tumoral tissues from subjects with HCV-associated HCC delivers novel molecular signatures for determining RFS, especially among the late-recurrence subjects. The gene expression predictor may hold important insights into the pathophysiology of HCC recurrence and de novo tumor formation in cirrhotic patients (2). When gene expression profiles from HepG2 cells expressing either T or NT core protein were compared, only one gene—CSNK1A1—was found to be up-regulated both in T and NT. This gene was more highly expressed in NT than T (in Tables, it seems to be the contrary). CSNK1, encoding the Ser/Thr kinase casein kinase I epsilon, was suspected of playing a role in cancer because loss-of-function mutations cause excess tissue growth (27, 28). Transcription regulation, signal transduction and participating in lipid metabolism genes were up-regulated both in T and NT, but PINX1-telomerase dependent telomere's maintenance (28, 29) that can promote cell growth is up-regulated in NT expressing cells. FLJ32642 related with liver cancer and CLCN5 related with resistance to causative organism infection were obvious up-regulated in T expressing cells, which might be the reason why in tumoral tissue the ability of inflammatory reaction and pathogen clearance decreases (29-31). In NT, negative gene-MBD2 (31, 32) and genes participating in phosphatide synthetic gene were obviously down-regulated, which might be the important reason why core protein of some HCV strains causes the disorder of fat metabolism after HCV infection. Different gene expression profiles were found between

cells expressing T and NT, which could explain why patients infected with different HCV genotypes, even with different quasispecies of the same genotype might have different clinical features, and different prognosis after therapy (10). The function of 42.5% of genes modulated by core proteins was unknown, suggesting that the pathogenesis of HCV core protein still needs further study to determine the role of these genes. Our previous studies revealed that the core protein from different HCV strains had some discrepancy in their gene sequences and functions. For examples, the rate of apoptosis in HepG2 and Chang liver cells (14, 15) is different. In this study, the anti-apoptosis gene-BIRC4 was down-regulated in T. Up- and down-regulation genes participating in fat metabolism were found both in T and NT, which confirmed that core protein can combine with apolipoprotein and interfere with fat metabolism, but our result has some discrepancy with others (33-40); we found no down-regulated genes in NT, which might be caused by the fact 1) that different arrays were used in different research. Because different arrays contain different genes, and the detection sensitivity of different arrays are also different; 2) that the structure and functions of core protein from different genotypes or different quasispecies used in each research have some difference; 3) that expression plasmids were different; 4) that different cell lines were used; and 5) that the time of extraction of cell mRNA after transfection were different. In this investigation, we selected two quasispecies Core of HCV genotype 1b from the HCV-related HCC tumoral tissue (T) and non-tumoral tissue (NT) (late stage of HCV infection). Affimetrix U133 Plus2.0 was used to investigate and compare the effect of these two core proteins on HepG2 cell gene expression profiling. Our results showed that HCV core proteins isolated from different quasispecies may play different roles in host cell metabolism since gene expression profiles in the HepG2 cells expressing different core proteins were implicated in pathogenesis/oncogenesis, which is helpful for our understanding of the biological functions of different quasispecies of HCV genotype 1b core protein.

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None declared.

Conflict of interest

None declared.

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