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Microarray analyses and molecular profiling of Stat3 signaling pathway induced by hepatitis C virus core protein in human hepatocytes

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Abstract

Hepatitis C virus (HCV) infection is a major contributor to the development of end-stage liver disease, including cirrhosis and hepatocellular carcinoma (HCC). We have previously shown that HCV core protein promotes immortalization of primary human hepatocytes. To identify molecular changes involved in core protein-mediated immortalization, we have investigated differential gene expression by microarray analyses in primary human hepatocytes and HCV core gene introduced hepatocytes after senescence (early passage), immortalization (middle passage), and anchor-independent growth (late passage). Out of 33,000 human genes screened, 1918 transcripts were differentially expressed (>2-fold) in immortalized human hepatocytes (IHH) as compared to negative controls. Our analyses provided a molecular portrait of changes in gene expression associated with three distinct stages of hepatocytes after introduction of HCV core gene. Many of the overall changes were involved with important cellular pathways, including cell growth regulation, immune regulation, oxidative stress, and apoptosis. We focused on the Stat3 signaling pathway by further verifying selected genes at the protein level relevant to hepatocyte growth regulation. Our data suggested that the introduction of HCV core protein results in an increase in expression of IL-6, gp130, leptin receptor, and Stat3. Upregulation of these genes in turn may regulate c-*myc* and cyclin D1, downstream of the Stat3 signaling pathway. Identification of these modulated genes with potential roles may help in the selection of targets for therapies against HCV-mediated liver disease progression.

Keywords: Hepatitis C virus; Core protein; Immortalized human hepatocytes; Microarray analyses; Interleukin-6; Stat3 signaling pathway

Introduction

HCC is one of the most common malignancies worldwide and is associated with multiple risk factors (Bosch et al., 2003). HCC is believed to arise from preneoplastic lesions, usually in the background of cirrhosis (Suriawinata and Xu, 2004). The etiological association between HCV infection and the development of HCC has been established (Alter, 1995; Koike et al., 2000; Saito et al., 1990); however, the molecular mechanisms of hepatocarcinogenesis are not well understood. HCV core protein has been detected in various subcellular compartments, including cytosol, lipid droplets, endoplasmic reticulum/Golgi apparatus, mitochondria, and nuclei (Lai and Ware, 2000). This broad intracellular distribution raises the possibility that core protein may modulate multiple cellular processes. In vitro studies have shown that HCV core protein transcriptionally regulates cellular promoters, and plays an inhibitory role against apoptosis under certain conditions (Ray and Ray, 2001; Bergqvist and Rice, 2001; Lai and Ware, 2000). Expression of core protein leads to the development of progressive hepatic steatosis, and HCC in transgenic mice (Moriya et al., 1997, 1998). Hepatic steatosis occurs at a high rate in chronic hepatitis C patients, and a close relationship between steatosis and intrahepatic core protein expression has been noted (Fujie et al., 1999). Overproduction and release of

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HCV nucleocapsid into the blood stream, and an accumulation of core protein in the liver of infected chimpanzees in the acute phase of infection have been suggested (Maillard et al., 2001). We have observed that HCV core protein promotes the immortalization of primary human hepatocytes, a natural host for virus replication and tropism (Ray et al., 2000). IHH displayed a weak level of core protein expression and continuous growth for more than 5 years. Telomerase activity, a characteristic of transformed cells, was observed in HCV core-transfected hepatocytes after senescence. Expression of anti-sense core gene in IHH induced cell death (Basu et al., 2002). These results suggested that the hepatocytes, during the early stage of immortalization, require core protein for survival. Anchorage-independent growth of IHH provided further evidence for a transformed phenotype at later passage levels.

Although immortalization of primary human hepatocytes (Ray et al., 2000) or direct induction of HCC in transgenic mice (Moriya et al., 1998) by HCV core protein have been demonstrated, the molecular mechanism of HCV core protein-induced hepatocyte growth regulation remains unknown. Analysis of hepatocyte populations from HCV-infected patients, at various stages en route to malignancy, would be the direct approach to understand the cellular and molecular processes of HCV-mediated HCC. However, primary cultures of HCV-infected hepatocytes from liver at various neoplastic stages are difficult to establish, and no cell lines at the intermediate stages of neoplastic transformation are available for mechanistic studies. Therefore, HCV core-transfected primary human hepatocytes at different stages, en route to immortalization and transformation, were used as a model to gain insight into modulation of candidate genes or novel pathways by DNA microarray analysis. Our results suggested modulation of a number of cellular genes with apoptosis/cell growth regulation, cytokine expression, and a variety of other cellular pathways.

Results

Altered gene expression following introduction of HCV core into primary human hepatocytes

The goal of our study was to identify changes in gene expression of primary human hepatocytes associated with different stages post immortalization by HCV core protein. We have utilized the Human 133 Plus 2.0 expression GeneChip microarray from Affymetrix containing 33,000 well-substantiated human genes. Data from microarray were analyzed and compared with primary human hepatocytes by Affymetrix software, and filtered using the Affymetrix Data Mining tool. Approximately 1918 genes were differentially expressed in core introduced hepatocytes as compared to primary human hepatocytes. A complete list of the genes with symbol, probe set ID, accession number, and functional description are provided as Supplementary Table 1. Of these, 736, 749, and 770 genes were upregulated (>2-fold) in the early, middle, and late passage hepatocytes, respectively. Genes whose expression were downregulated (>2-fold) following core protein expression represented 575, 606, and 522 in the early, middle, and late passage hepatocytes. A detailed profile analysis led us to group the genes into 9 clusters based upon their functions (Table 1). Genes were clustered using Spotfire software and a heat map with two-dimensional hierarchical clustering. The map illustrates a gradual change in gene expression between normal human hepatocytes and IHH (Fig. 1). Many of the overall changes were involved with important cellular pathways, including cytokine responsive genes, apoptosis, oncogenes, protein kinase cascade, lipid metabolism related genes, oxidative stress related genes, and immunomodulatory genes (Supplementary Tables 2–8). A detailed analysis of all genes exhibiting changes in expression is beyond the scope of the current report. We particularly focused on selected genes relevant to hepatocyte growth regulation, stress response, and cytokine modulation to further understand HCV core protein mediated functions.

HCV core protein activates Stat3 in human hepatocytes

Signal transducers and activators of transcription (Stat) family proteins function as the downstream effectors of cytokine signaling, and play a critical role in cell growth regulation. Among these, Stat3 is often constitutively activated in many cancers, including those involving the liver. This activation has been associated with proliferation and antiapoptotic responses (Bowman et al., 2000). Stat3 is activated by multiple cytokines, including IL-6, LIF, and OSM, using the gp130 receptor. Our microarray analyses suggested an upregulation of IL-6 and other

Table 1
Detailed profile analysis by clustering of genes from microarray

Cluster no.	Functions involved	No. of genes	Upregulated			Downregulated		
			Early	Middle	Late	Early	Middle	Late
1	Apoptosis	44	14 (31.8%)	18 (40.9%)	10 (22.7%)	9 (20.5%)	11 (25%)	22 (50%)
2	Oxidative stress	6	1 (16.7%)	1 (16.7%)	0 (0%)	5 (83.3%)	5 (83.3%)	5 (83.3%)
3	Lipid metabolism	19	5 (26.3%)	4 (21%)	5 (26.3%)	8 (42.1%)	12 (63.15%)	12 (63.15%)
4	Signal transduction	202	87 (43.1%)	83 (41.1%)	83 (41.1)	61 (30.2%)	66 (32.67%)	44 (21.78%)
5	Protein kinase cascade	25	9 (36%)	7 (28%)	8 (32%)	9 (36%)	8 (32%)	9 (36%)
6	Oncogenesis	17	5 (29.4%)	5 (29.4%)	6 (35.2%)	6 (35.29%)	4 (23.53%)	4 (23.53%)
7	Cell cycle	65	30 (46%)	35 (53.8%)	30 (46%)	13 (20%)	9 (13.84%)	9 (13.84%)
8	Immune regulation	54	27 (50%)	20 (37%)	16 (29.6%)	16 (29.63%)	16 (29.63%)	16 (29.63%)
9	Cytokine response	12	7 (58.3%)	5 (41.6%)	4 (33.3%)	4 (33.33%)	2 (16.66%)	3 (25%)