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Altered Levels of Cytochrome P450 Genes in Hepatitis B or C Virus-infected Liver Identified by Oligonucleotide Microarray

NORIO IIZUKA[1,2], MASAAKI OKA[1], YOSHIHIKO HAMAMOTO[3], NAOHIDE MORI[1], TAKAO TAMESA[1], AKIRA TANGOKU[1], TAKANOBU MIYAMOTO[3], SHUNJI UCHIMURA[3], HIRONOBU NAKAYAMA[4], KENJI HAMADA[4] and HISAFUMI YAMADA-OKABE[4]

[1]Department of Surgery II and [2]Department of Bioregulatory Function, Yamaguchi University School of Medicine, 1-1-1 Minami-kogushi, Ube, Yamaguchi 755-8505; [3]Department of Computer Science and Systems Engineering, Faculty of Engineering, Yamaguchi University, 2-16-1 Tokiwadai, Ube, Yamaguchi 755-8611; [4]Pharmaceutical Research Department 4, Kamakaura Research Laboratories, Chugai Pharmaceutical Co. Ltd., 200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan

Abstract. *Background: Molecular pathogenesis of hepatocellular carcinoma (HCC) remains to be clarified. Many studies with DNA chip technology have revealed altered levels of several cytochrome P450 (CYP) family genes in human HCC. However, little is known about their alterations in hepatitis B virus (HBV)- and hepatitis C virus (HCV)-infected livers. Materials and Methods: We here used high-density oligonucleotide arrays to evaluate alterations of CYP genes in five of each HBV- or HCV-infected livers in comparison with six normal livers. We extracted the data of 32 CYP genes from those of 12600 genes. Results: Among these 32 CYPs, expression levels of four genes were insignificant. The expressions of CYP1B1 and CYP3A7 were up-regulated, whereas the expressions of 12 other CYPs, including CYP2A6, CYP2A7 and CYP2C19, were down-regulated in HBV- and/or HCV-infected livers compared with normal livers. Conclusion: These data will allow us to better understand the roles of CYPs in the pathogenesis of HBV- and HCV-related HCCs.*

Hepatocellular carcinoma (HCC) is a common cause of cancer death throughout the world (1,2). Chronic infection with hepatitis B or C virus (HBV or HCV) is the most clearly established risk factor for HCC. The majority of HCC can be attributed to infection with either of the two viruses (1,2). However, the genetic basis underlying these diseases remains unclear. To overcome this dilemma, many researchers have applied DNA chip technology to elucidate genome-wide changes in these diseases (3-13). Interestingly, as summarized in Table I, commonly found in those studies are altered levels of several cytochrome P450 family genes (*CYPs*), such as *CYP2E*, *CYP2A6* and *CYP2A7*, in HCC *versus* non-cancerous liver.

The cytochrome P450 system is a group of enzymes that are responsible for metabolizing many endogenous and exogenous substances including xenobiotics or carcinogens into more hydrophilic substances (14). This means that altered levels of *CYPs* might be related to hepatocarcinogenesis. Some types of *CYPs* are likely to be involved in rat hepatocarcinogenesis (15). It has also been shown that the genetic polymorphisms of *CYP2E*, *CYP2D6* and *CYP2C19* are associated with the development of HCC (16, 17). However, it remains unclear how these *CYPs* are involved in HBV- or HCV-related hepatocarcinogenesis. To elucidate their roles in these diseases, it is essential to clarify expression patterns of *CYP* genes in HBV- or HCV-infected livers that represent hypercarcinogenic states for HCC. For this purpose, we tested oligonucleotide microarrays with RNA isolated from human livers that were uninfected or infected with HBV or HCV, and we identified *CYP* genes that were differentially expressed between uninfected and infected liver tissues. In combination with gene expression profiles of *CYPs* in HCC (3-13), the possible roles in pre-cancerous livers are discussed.

Materials and Methods

Patients. Non-tumourous liver samples were obtained from six patients who underwent hepatic resection for benign or metastatic liver tumours. All six patients showed liver function values within normal limits. Their livers were histologically normal and were

Abbreviations: CYP, cytochrome P450; HCV, hepatitis C virus; HCC, hepatocellular carcinoma.

Correspondence to: Prof. Masaaki Oka, Department of Surgery II, Yamaguchi University School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan. Tel/Fax:+81 836 22 2262, e-mail: 2geka-1@po.cc.yamaguchi-u.ac.jp

Key Words: DNA chip, cytochrome P450, HBV, HCV, hepatocellular carcinoma.

Table I. *CYPs expressed differentially in human hepatocellular carcinoma.*

Cytochrome p450 (CYP)	Condition	Type of HCC	Ref.
CYP2C, CYP2E	down-regulation in tumour *vs.* normal liver	HBV-associated HCC	Lau *et al.*, 2000 (Ref.3)
CYP2C	down-regulation in tumour *vs.* normal liver	HCC*	Shirota *et al.*, 2001 (Ref.4)
CYP2B, CYP2C, CYP17	down-regulation in tumour *vs.* normal liver	HBV-associated HCC	Xu *et al.*, 2001 (Ref.5)
CYP2C9, CYP2E, CYP4F2	down-regulation in tumour *vs.* normal liver	HCC**	Xu *et al.*, 2001 (Ref.6)
CYP2A6, CYP2C8, CYP2C9 *CYP2E*	down-regulation in tumour *vs.* normal liver down-regulation in HBV-associated HCC	HCC*	Okabe *et al.*, 2001 (Ref.7)
CYP2A6, CYP2B, CYP8B1	down-regulation in tumour *vs.* normal liver	HCC**	Tackels-Horne *et al.*, 2001(Ref.8)
CYP2A7, CYP2E *CYP2A7, CYP2E*	down-regulation in tumour *vs.* normal liver down-regulation in tumour *vs.* normal liver	HBV-associated HCC HCV-associated HCC	Iizuka *et al.*, 2002 (Ref.9)
CYP2C8, CYP27A1	up-regulation in tumour *vs.* normal liver	HCC**	Li *et al.*, 2002 (Ref.10)
CYP3A3, CYP27A1	down-regulation in tumour with venous invasion	HCC**	Chen *et al.*, 2002 (Ref.11)
CYP2A7	down-regulation in metastatic HCC	HBV-associated HCC	Cheung *et al.*, 2002 (Ref.12)
CYP2A6, CYP2A7, *CYP2A13, CYP2C8*	down-regulation during tumour progression	HCC*	Chuma *et al.*, 2003 (Ref.13)

*Samples consisting of HBV- and HCV-associated HCCs, ** Sample labels are unclear

seronegative for both HBs antigen and HCV antibody. Five HBV-infected and five HCV-infected liver samples were obtained from the non-tumourous areas of ten patients with hepatocellular carcinoma. Among the ten liver samples, four were histopathologically diagnosed as chronic hepatitis and six as liver cirrhosis. All sixteen patients enrolled in this study underwent uniform premedication before surgery and general anesthesia with 1.7% sevoflurane and 60% nitrous oxide. None of them underwent specific therapies. Informed consent in writing was obtained from all patients before surgery. The study protocol was approved by the Institutional Review Board for Human Use of the Yamaguchi University School of Medicine, Japan.

Oligonucleotide microarray and gene selection. Extraction of RNA, syntheses of cDNA and cRNA, and oligonucleotide microarray screening were performed as described previously (9, 18, 19). In the present study, to obtain expression data of many *CYPs*, large-size DNA chips (huU95A DNA chips; Affymetrix, Santa Clara, CA, USA) containing 12600 genes were used for microarray experiments. We extracted the data of 32 *CYPs* from those of 12600 genes. For statistical analysis of gene expression, ANOVA with Fisher's projected least significant difference (PLSD) test was performed with the use of StatView 5.0 software (Abacus Concepts, Berkeley, CA, USA). A $p<0.05$ was judged as significant.

Results

We examined the differential expression of 32 *CYP* genes (Figure 1 and Table II) on the basis of the oligonucleotide microarray data. Levels of expression of four genes were very low in normal, HBV-positive and HCV-positive livers (average of arbitrary expression units was less than 40 by Affymetrix) and were thus considered insignificant. Levels of the remaining 28 genes were higher than those of the four genes and were considered biologically significant. Of these 28, levels of *CYP1B1* and *CYP3A7* were up-regulated and levels of 12 *CYP* genes, including *CYP2A* subfamily (*2A4*, *2A6* and *2A7*) and *CYP2C19* were down-regulated in HBV- and/or HCV-infected livers compared with HBV-/HCV-double-negative livers. Levels of *CYP3A7* were significantly higher in HCV-infected liver than in HBV-infected and HBV-/HCV-double-negative livers. Levels of *CYP27A1* were significantly lower in HBV-infected liver than in HCV-infected and HBV-/HCV-double-negative livers. Expression levels of the remaining 14 *CYP* genes were unchanged by either HBV or HCV infection.

Discussion

Many studies have revealed the relationship between *CYPs* and human hepatocarcinogenesis (17,20,21). Chau *et al.* showed that poor metabolizer phenotype caused by the mutation of the *CYP2C19* gene in cirrhotic patients with HCV infection is associated with a high risk for developing HCC (17). *CYP27A*, which is known as a D3-25 hydroxylase, was found to be significantly up-regulated in HCC (20). *CYP1A1* polymorphism is an important modulator of the hepatocarcinogenic effect of tobacco-derived polycyclic aromatic hydrocarbons (21). Given the fact that *CYPs* are large family genes consisting of many

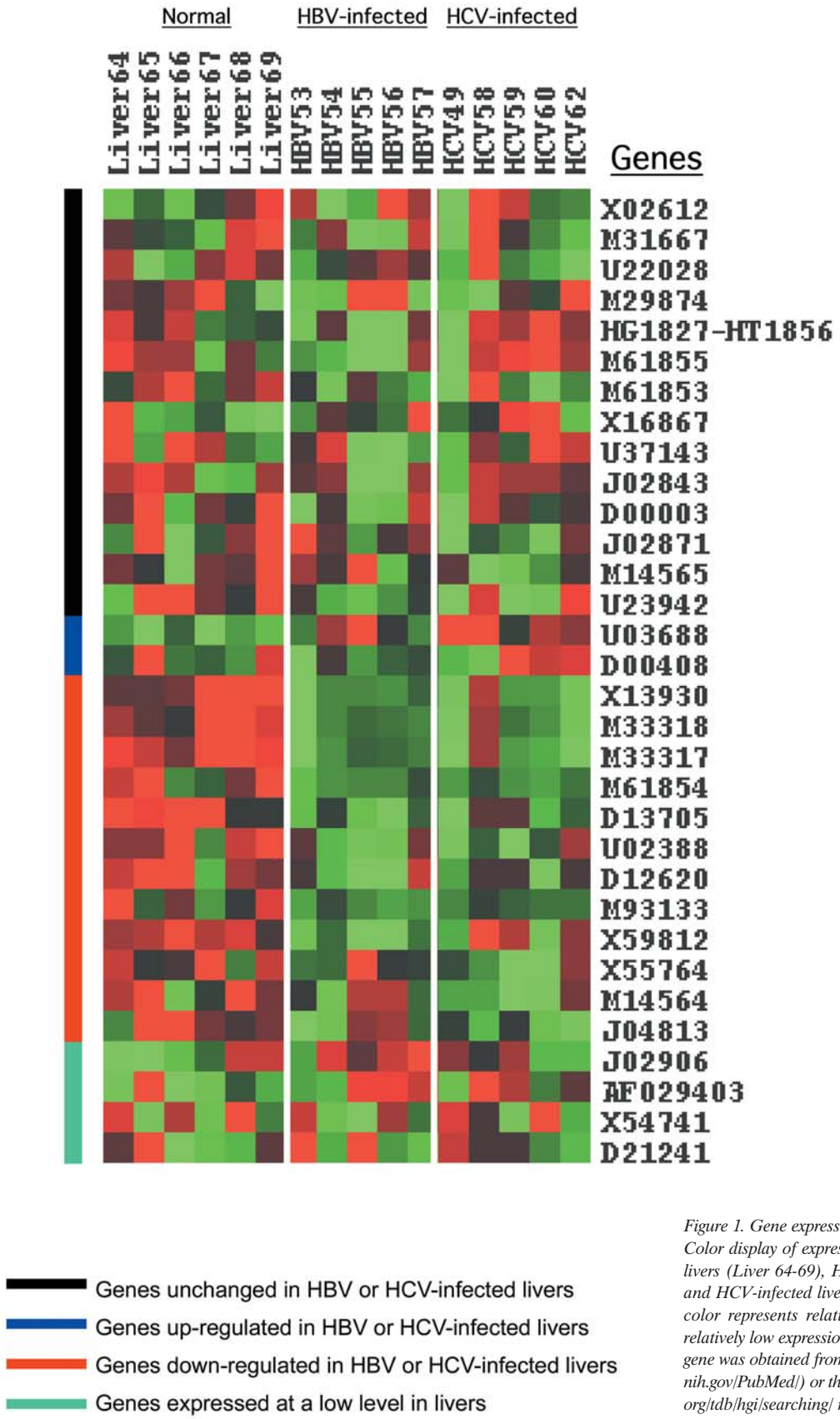

Figure 1. Gene expression patterns of the 32 CYP genes. Color display of expression of 32 CYP genes in normal livers (Liver 64-69), HBV-infected livers (HBV 53-57) and HCV-infected livers (HCV 49, 58-60 and 62). Red color represents relatively high expression and green relatively low expression. The accession number of each gene was obtained from PubMed (http://www3.nibi.nlm.nih.gov/PubMed/) or the TIGR database (http://www.tigr.org/tdb/hgi/searching/ reports.html).

Table II. *Summary of the 32 CYP genes expressed in human livers.*

GB number	Gene name (symbol)	Normal liver	HBV-infected liver	HCV-infected liver	Locus	Representative substrates
X02612	*CYP1A1*	164±38	203±70	182±52	15q22-q24	NSAIDs, Polycyclic aromatic hydrocarbons
M31667	*CYP1A2*	766±159	531±180	614±265	15q22-qter	Acetaminophen, Paracetamol
U22028	*CYP2A13*	1452±278	1455±162	1328±483	19q13.2	Dimethylaniline, N-Nitrosomethylphenylamine
M29874	*CYP2B6*	11803±1083	11138±1720	10786±1274	19q13.2	Many NSAIDs, Erythromycin, Ketamine
HG1827-HT1856	*P450 (subfamily IIc)*	15974±883	12764±1435	16844±2335		unknown
M61855	*CYP2C9*	8560±440	7109±563	8967±678	10q24	Acetaminophen, Paracetamol, Aceclofenac
M61853	*CYP2C18*	1097±92	778±88	846±254	10q24	Many NSAIDs, Imipramine, Cyclophosphamide
X16867	*P450-IID (clone pMP34)*	2943±629	4107±779	3955±631	8q21	unknown
U37143	*CYP2J2*	1371±104	1142±147	1380±105	1p31.3-p31.2	Arachidonic acids
J02843	*CYP2E*	25088±2961	18274±5179	25221±2460	10q24.3-qter	Ethanol, Acetone, Benzene, Nitrosoamine, *etc*.
D00003	*CYP3A3*	17943±1646	13779±1786	15135±1826	7q22.1-q31.33	Niphedipine, *etc*.
J02871	*CYP4B1*	661±80	682±61	515±62	1p34-p12	Fatty acids, Arachidonic acids, Bezene, Styrene, *etc*.
M14565	*CYP11A*	275±32	293±32	232±21	15q23-q24	Cholesterol
U23942	*CYP51*	593±66	406±37	422±101	7q21.2-q21.3	Sterol
U03688	*CYP1B1*	58±8	104±18*	118±9*	2p21	Many Polycyclic aromatic hydrocarbons
D00408	*CYP3A7*	4801±422	5047±477	8389±1387*,**	7q21-q22.1	Many NSAIDs, Testosterone, Aflatoxin B1
X13930	*P450-IIA4 (=CYP2A7)*	7344±915	2781±522*	2955±976*	19q13.2	unknown
M33318	*CYP2A6*	15475±1807	7100±1138*	7598±1734*	19q13.2	Coumarin, Nicotin, Aflatoxin B1, Nitrosoamines
M33317	*CYP2A7*	9114±531	4253±703*	4109±1032*	19q13.2	unknown
M61854	*CYP2C19*	1074±301	323±65*	350±55*	10q24.1-q24.3	Xenobiotics, Polycyclic aromatic hydrocarbons, Hormones
D13705	*CYP4A11*	2638±143	1882±105*	1971±173*	1p33	Retinoic acid (All trans-), Fatty acids, Arachidonic acids
U02388	*CYP4F2*	2842±212	2156±174*	2147±200*	19pter-p13.11	Eicosanoids such as Leukotoriene B4, Ebastine
D12620	*CYP4F3*	4700±343	3492±442*	3762±269	19p13.2	Eicosanoids such as Leukotoriene B4, Fatty acids
M93133	*CYP7A1*	433±188	69±33*	134±22	8q11-q12	Several Hydroxycholesterols
X59812	*CYP27A1*	2297±75	1049±156*	2006±241**	2q33-qter	Nutritional agents and Vitamins
X55764	*CYP11B1*	242±30	208±36	113±48*	8q21	Glucocorticoid and Mineralocorticoid
M31153	*CYP17A1*	195±62	121±48	16±44*	10q24.3	Corticosteroids, Sex hormones, NSAIDs
J04813	*CYP3A5*	6715±1149	3973±1048	3154±658*	7q21	Niphedipine, Cyclosporine, Many Steroids
J02906	*CYP2F1*	<0	58±57	<0	19q13.1-q13.2	Toxins derived from the Fermentation of Tryptophan
AF029403	*CYP7B1*	19±22	55±21	47±18	8q21.3	Glucocorticoid and Mineralocorticoid
X54741	*CYP11B2*	<0	<0	<0	8q21-q22	Corticosteroids
D21241	CYP19	<0	<0	<0	15q21.1	Sex hormones, Several chemicals

*, $p<0.05$ compared with normal liver by ANOVA with Fisher's PLSD test. **, $p<0.05$ compared with HBV-infected liver by ANOVA with Fisher's PLSD test. The accession number of each gene was obtained from PubMed (http://www3.nibi.nlm.nih.gov/PubMed/) or the TIGR database (http://www.tigr.org/tdb/hgi/searching/reports.html). Symbol used is based on the data from LocusLink (http://www.nibi.nlm.nih.gov/ LocusLink/).

isoforms, high-throughput technology is needed to elucidate their individual performance in hepatocarcinogenesis. Recent studies with DNA chip technology have gradually disclosed significant changes of some *CYP* mRNA levels in HCC (3-13). However, there had so far been no studies that evaluated expression profiles of *CYPs* in HBV- and HCV-infected livers, both of which represent the hypercarcinogenic state for HCC. We therefore focused on expression profiles of *CYPs* in HBV- and HCV-infected livers in the present study.

Among the *CYP2A* family of genes, we found that mRNA levels of *CYP2A6* and *CYP2A7* were decreased in both HBV- and HCV-infected livers in comparison with control livers. Previous studies showed their down-regulation in HCC in comparison with normal liver (7-9). Our present data provide an additional finding that expression levels of these two genes are down-regulated in HBV- or HCV-infected liver. *CYP2A6* is involved in the metabolism of hepatic carcinogens such as aflatoxin B1 and nitrosoamine, and it is found to be regulated transcriptionally by hepatocyte nuclear factor 4 (HNF4), which has shown activating effects on particular *CYP* promoters (22). In contrast, the role of *CYP2A7* remains to be elucidated. Several *CYPs* were also found to be down-regulated by nitric oxide and interleukin 6 (23,24). These studies raise the possibility that down-regulation of *CYP2A6* and *CYP2A7* may be related to inflammation caused by HBV and HCV infection. Chuma *et al.* provided unique evidence that expression levels of *CYP2A6* and *CYP2A7* were decreased during tumour progression of HCC (13). Cheung *et al.* also reported that *CYP2A7* was down-regulated in metastatic HCC in comparison with primary HCC (12). Collectively, these studies suggest that down-regulation of *CYP2A6* and *CYP2A7* may represent withdrawal of the normal function of hepatocytes during the transition from chronic inflammation to development of HCC. More interestingly, it is known that HCV core peptide (position 178-187) shows sequence homology with CYP2A6 and CYP2A7. With this fact in mind, Kammer *et al.* showed the possibility that cytotoxic T cells induced by the HCV peptide recognize CYP2A6 and CYP2A7 and such an immune response is related to the pathogenesis of this disease (25). Thus, in the case of HCV-infected liver, down-regulation of *CYP2A6* and *CYP2A7* may be an immunologically crucial step in the development of the disease.

Among the *CYP2C* family of genes, mRNA levels of *CYP2C9* and *CYP2C18* were not affected by HBV and HCV infection in our series. In contrast, we found that *CYP2C19* was significantly down-regulated in HBV- and HCV-infected livers, even though it is located close to *CYP2C9* and *CYP2C18* on chromosome 10q24. CYP2C19 metabolizes many polycyclic aromatic hydrocarbons, xenobiotics and sex hormones. Chau *et al.* showed the relationship between the genetic polymorphism of the *CYP2C19* gene and a high risk of developing HCC (17). In addition to the genetic aberration, our present result suggests that decreased expression of the *CYP2C19* transcript following HBV and HCV infections may promote hepatocarcinogenesis.

CYP2E, the ethanol-inducible form, is of interest because of its ability to metabolize and activate many toxicologically important substrates including ethanol, carbon tetrachloride, acetaminophen and nitrosoamine to more toxic products (26). Several studies, including ours, have shown down-regulation of *CYP2E* in HCC tissues (3,6,7,9). Our present data shows that *CYP2E* transcript levels in non-cancerous liver are not affected by HBV or HCV infections. This result suggests that levels of *CYP2E* transcript can be down-regulated only in transformed cancer cells. Thus, withdrawal of hepatic *CYP2E* may be related to cancer progression rather than carcinogenesis.

We found down-regulation of *CYP17A1*, a metabolizer for corticosteroids and sex hormones, in HCV-infected liver *versus* control liver. A recent study revealed the relationship between CYP17 polymorphisms and breast cancer risk (27). Yu *et al.* showed that CYP17A1 genotype increased the risk of HBV-related HCC in the presence of some genotype of androgen receptor (28). However, its relation to HCV-related hepatocarcinogenesis remains unknown. Increased levels of CYP3A7 in HCV-infected liver and decreased levels of CYP27A1 in HBV-infected liver suggest virus-type specific pathogenesis for HCC; however, to our knowledge, there were no reports evaluating the relationship of the 2 genes to the biology of HBV and HCV. Thus, further studies are needed to elucidate how their altered levels are related to carcinogenesis caused by the two hepatitis viruses.

It is well known that single nucleotide polymorphisms (SNPs) in the coding or promoter regions of *CYP* genes can affect the metabolizing activities (29,30), suggesting that additive analyses of the protein activities are needed. Thus, the main limitation of our present study is an analysis of transcriptome of hepatic *CYPs*. Further analyses of the protein levels and activities of hepatic CYPs are necessary to gain new insights into our present results; nevertheless, our present finding that many *CYPs* were down-regulated in HBV- and HCV-infected livers will enable us to better understand human hepatocarcinogenesis

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References

1 Okuda K: Hepatocellular carcinoma. J Hepatol *32*: 225-237, 2000.
2 Schafer DF and Sorrell MF: Hepatocellular carcinoma. Lancet *353*: 1253-1257, 1999.
3 Lau WY, Lai PB, Leung MF, Leung BC, Wong N, Chen G, Leung TW and Liew CT: Differential gene expression of hepatocellular carcinoma using cDNA microarray analysis. Oncol Res *12*: 59-69, 2000.

4 Shirota Y, Kaneko S, Honda M, Kawai HF and Kobayashi K: Identification of differentially expressed genes in hepatocellular carcinoma with cDNA microarrays. Hepatology *33*: 832-840, 2001.
5 Xu XR, Huang J, Xu ZG, Qian BZ, Zhu ZD, Yan Q, Cai T, Zhang X, Xiao HS, Qu J, Liu F, Huang QH, Cheng ZH, Li NG, Du JJ, Hu W, Shen KT, Lu G, Fu G, Zhong M, Xu SH, Gu WY, Huang W, Zhao XT, Hu GX, Gu JR, Chen Z and Han ZG: Insight into hepatocellular carcinogenesis at transcriptome level by comparing gene expression profiles of hepatocellular carcinoma with those of corresponding noncancerous liver. Proc Natl Acad Sci USA *98*: 15089-15094, 2001.
6 Xu L, Hui L, Wang S, Gong J, Jin Y, Wang Y, Ji Y, Wu X, Han Z and Hu G: Expression profiling suggested a regulatory role of liver-enriched transcription factors in human hepatocellular carcinoma. Cancer Res *61*: 3176-3181, 2001.
7 Okabe H, Satoh S, Kato T, Kitahara O, Yanagawa R, Yamaoka Y, Tsunoda T, Furukawa Y and Nakamura Y: Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression. Cancer Res *61*: 2129-2137, 2001.
8 Tackels-Horne D, Goodman MD, Williams AJ, Wilson DJ, Eskandari T, Vogt LM, Boland JF, Scherf U and Vockley JG: Identification of differentially expressed genes in hepatocellular carcinoma and metastatic liver tumors by oligonucleotide expression profiling. Cancer *92*: 395-405, 2001.
9 Iizuka N, Oka M, Yamada-Okabe H, Mori N, Tamesa T, Okada T, Takemoto N, Tangoku A, Hamada K, Nakayama H, Miyamoto T, Uchimura S and Hamamoto Y: Comparison of gene expression profiles between hepatitis B virus- and hepatitis C virus-infected hepatocellular carcinoma by oligonucleotide microarray data on the basis of a supervised learning method. Cancer Res *62*: 3939-3944, 2002.
10 Li Y, Li Y, Tang R, Xu H, Qiu M, Chen Q, Chen J, Fu Z, Ying K, Xie Y and Mao Y: Discovery and analysis of hepatocellular carcinoma genes using cDNA microarrays. J Cancer Res Clin Oncol *128*: 369-379, 2002.
11 Chen X, Cheung ST, So S, Fan ST, Barry C, Higgins J, Lai KM, Ji J, Dudoit S, Ng IO, Van De Rijn M, Botstein D and Brown PO: Gene expression patterns in human liver cancers. Mol Biol Cell *13*: 1929-1939, 2002.
12 Cheung ST, Chen X, Guan XY, Wong SY, Tai LS, Ng IO, So S and Fan ST: Identify metastasis-associated genes in hepatocellular carcinoma through clonality delineation for multinodular tumor. Cancer Res *62*: 4711-4721, 2002.
13 Chuma M, Sakamoto M, Yamazaki K, Ohta T, Ohki M, Asaka M and Hirohashi S: Expression profiling in multistage hepatocarcinogenesis: identification of HSP70 as a molecular marker of early hepatocellular carcinoma. Hepatology *37*: 198-207, 2003.
14 Rogers JF, Nafziger AN and Bertino JS Jr: Pharmacogenetics affects dosing, efficacy, and toxicity of cytochrome P450-metabolized drugs. Am J Med *113*: 746-750, 2002.
15 White IN and De Matteis F: The role of CYP forms in the metabolism and metabolic activation of HCFCs and other halocarbons. Toxicol Lett *124*: 121-128, 2001.
16 Silvestri L, Sonzogni L, De Silvestri A, Gritti C, Foti L, Zavaglia C, Leveri M, Cividini A, Mondelli MU, Civardi E and Silini EM: CYP enzyme polymorphisms and susceptibility to HCV-related chronic liver disease and liver cancer. Int J Cancer *104*: 310-317, 2003.
17 Chau TK, Marakami S, Kawai B, Nasu K, Kubota T and Ohnishi A: Genotype analysis of the CYP2C19 gene in HCV-seropositive patients with cirrhosis and hepatocellular carcinoma. Life Sci *67*: 1719-1724, 2000.
18 Iizuka N, Oka M, Yamada-Okabe H, Mori N, Tamesa T, Okada T, Takemoto N, Hashimoto K, Tangoku A, Hamada K, Nakayama H, Miyamoto, T, Uchimura S and Hamamoto Y: Differential gene expression in distinct virologic types of hepatocellular carcinoma: Association with liver cirrhosis. Oncogene *22*: 3007-3014, 2003.
19 Iizuka N, Oka M, Yamada-Okabe H, Nishida M, Maeda Y, Mori N, Takao T, Tamesa T, Tangoku A, Tabuchi H, Hamada K, Nakayama H, Ishitsuka H, Miyamoto T, Hirabayashi A, Uchimura S and Hamamoto Y: Oligonucleotide microarray for prediction of early intrahepatic recurrence of hepatocellular carcinoma after curative resection. Lancet *361*: 923-929, 2003.
20 Gascon-Barre M, Demers C, Ghrab O, Theodoropoulos C, Lapointe R, Jones G, Valiquette L and Menard D: Expression of CYP27A, a gene encoding a vitamin D-25 hydroxylase in human liver and kidney. Clin Endocrinol *54*: 107-115,2001.
21 Yu MW, Chiu YH, Yang SY, Santella RM, Chern HD, Liaw YF and Chen CJ: Cytochrome P450 1A1 genetic polymorphisms and risk of hepatocellular carcinoma among chronic hepatitis B carriers. Br J Cancer *80*: 598-603, 1999.
22 Jover R, Bort R, Gomez-Lechon MJ and Castell JV: Cytochrome P450 regulation by hepatocyte nuclear factor 4 in human hepatocytes: a study using adenovirus-mediated antisense targeting. Hepatology *33*: 668-675, 2001.
23 Hara H and Adachi T: Contribution of hepatocyte nuclear factor-4 to down-regulation of CYP2D6 gene expression by nitric oxide. Mol Pharmacol *61*: 194-200, 2002.
24 Jover R, Bort R, Gomez-Lechon MJ and Castell JV: Down-regulation of human CYP3A4 by the inflammatory signal interleukin-6: molecular mechanism and transcription factors involved. FASEB J *16*: 1799-801, 2002.
25 Kammer AR, van der Burg SH, Grabscheid B, Hunziker IP, Kwappenberg KM, Reichen J, Melief CJ and Cerny A: Molecular mimicry of human cytochrome P450 by hepatitis C virus at the level of cytotoxic T cell recognition. J Exp Med *190*: 169-176, 1999.
26 Cederbaum AI, Wu D, Mari M and Bai J: CYP2E1-dependent toxicity and oxidative stress in HepG2 cells. Free Radic Biol Med *31*: 1539-1543, 2001.
27 Wu AH, Seow A, Arakawa K, Van Den Berg D, Lee HP and Yu MC: HSD17B1 and CYP17 polymorphisms and breast cancer risk among Chinese women in Singapore. Int J Cancer *104*: 450-457, 2003.
28 Yu MW, Yang YC, Yang SY, Cheng SW, Liaw YF, Lin SM and Chen CJ: Hormonal markers and hepatitis B virus-related hepatocellular carcinoma risk: a nested case-control study among men. J Natl Cancer Inst *93*: 1644-1651, 2001.
29 Nebert DW: Suggestions for the nomenclature of human alleles: relevance to ecogenetics, pharmacogenetics and molecular epidemiology. Pharmacogenetics *10*: 279-290, 2000.
30 Pirmohamed M and Park BK: Genetic susceptibility to adverse drug reactions. Trends Pharmacol Sci *22*: 298-305, 2001.

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