Gene expression profile linked to *p53* status in hepatitis C virus-related hepatocellular carcinoma

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Received 27 August 2003; revised 10 November 2003; accepted 10 November 2003

First published online 26 November 2003

Edited by Takashi Gojobori

Abstract To clarify the role of *p53* in 22 hepatitis C virus (HCV)-infected hepatocellular carcinomas (HCCs), we compared the gene expression profiles of HCCs with wild-type *p53* (wt-p53) ($n=17$) and those with mutant-type *p53* (mt-p53) ($n=5$) by oligonucleotide microarray analysis. Among 83 *p53*-related genes identified by a supervised learning method, 25 were underexpressed, and 58 were overexpressed in mt-p53 HCCs compared with wt-p53 HCCs. With a computer search, we identified consensus p53-binding sequences in the 3-kb region upstream of the translation initiation site in 59 of the 83 genes, suggesting that the in vivo p53-associated transcription system is very complicated. These data will provide additional insights into p53-related pathogenesis in HCV-infected HCC.

Key words: p53; DNA chip; Gene expression; Hepatocellular carcinoma; Cancer

1. Introduction

Expression of the *p53* gene is a major marker for the genetic changes that occur in oncogenesis [1–4]. In particular, the p53 protein influences the transcription of many cellular genes by binding to DNA in a sequence-specific manner, resulting in inhibition of cell growth and invasion. Conversely, p53 mutants, which have been identified in a number of different human cancers, fail to bind to the consensus DNA binding site, resulting in a loss of tumor suppressor activity. Much research has been devoted to identifying genes and proteins involved in *p53* abnormalities [4]. More recently, several in vitro studies with DNA chip technology have identified gene expression patterns associated with *p53* status [5–7]. However, most of these studies have used cultured cells. Whether the patterns observed in cultured cells reflect those in human tissues remains unknown.

As in many cancers, abnormalities in the *p53* gene occur during the carcinogenesis and progression of hepatocellular carcinoma (HCC) [8]. However, it remains unclear how the HCC phenotype is influenced by *p53*. We have characterized several subgroups of human HCC with DNA chip technology [9–11]. In the present study, we investigated the gene expression profile of human HCC with respect to *p53* status with DNA microarrays. A role of *p53* abnormality has been documented in hepatitis B virus-related HCC [12] but not in hepatitis C virus (HCV)-related HCC. Therefore, we focused on *p53* abnormalities in HCV-related HCC. We identified a set of genes that are differentially expressed between HCCs with wild-type *p53* (wt-p53) and those with mutant-type *p53* (mt-p53).

2. Materials and methods

2.1. Patients

Twenty-two patients who underwent surgical treatment for HCC at Yamaguchi University Hospital between May 1997 and August 2000 were enrolled in this study. None of them underwent chemotherapy prior to surgery. Written informed consent was obtained from all patients before surgery. The study protocol was approved by the Institutional Review Board for Human Use at Yamaguchi University School of Medicine. A histopathologic diagnosis of HCC was made for all patients after surgery. All patients were positive for HCV antibody and were negative for hepatitis B virus surface antigen expression. Tumors were categorized on the basis of the International Union against Cancer TNM Classification [13] (Table 1).

2.2. Sample preparation

We divided all tumor specimens resected from each patient into two portions immediately after resection. One was fixed in 10% formalin and embedded in paraffin. The other was frozen in liquid nitrogen and stored at −80°C until oligonucleotide array analysis and cDNA sequencing.

2.3. cDNA sequencing of p53

RNA extraction and reverse transcription were performed as described previously [14]. We amplified a 722-bp fragment (exons 5–9) from *p53* cDNA by polymerase chain reaction (PCR) with primers 5′-GTTTCCGTCTGGGCTTCTTGCA-3′ (exon 4) and 5′-CAAG-

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Abbreviations: HCC, hepatocellular carcinoma; HCV, hepatitis C virus; wt-p53, wild-type p53; mt-p53, mutant-type p53; Gene abbreviations used are from LocusLink (http://www.ncbi.nlm.nih.gov/LocusLink/)

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)01345-0

Table 1
Summary of clinicopathologic factors and *p53* status

Sample no.	Age	Sex	TNM stage	Differentiation	Tumor size (cm)	*p53* status	Codon	Nucleotide change
HCV2T	49	M	IIIA	G2	13.5	Wild		
HCV6T	61	M	IIIA	G2	5	Wild		
HCV8T	75	M	I	G2	2.6	Wild		
HCV10T	73	M	IIIA	G2	8	Wild		
HCV18T	60	M	II	G2	3.2	Wild		
HCV19T	64	M	II	G3	2.1	Wild		
HCV21T	66	M	II	G3	1.8	Wild		
HCV22T	70	F	I	G2	4.8	Wild		
HCV23T	68	F	IIIA	G2	7.5	Wild		
HCV31T	67	F	I	G2	10.5	Wild		
HCV34T	66	M	II	G2	2.5	Wild		
HCV35T	72	F	IV	G3	23	Mutant	145	CTG→CGG (Leu→Arg)
HCV37T	64	M	II	G2	2.2	Mutant	193	CAT→CGT (His→Arg)
HCV42T	58	M	I	G1	2.5	Wild		
HCV43T	58	M	IV	G2	12	Mutant	244	GGC→GCC (Gly→Ala)
HCV45T	73	M	I	G2	2.8	Wild		
HCV49T	65	M	IIIA	G2	13	Mutant	244	GGC→GCC (Gly→Ala)
HCV59T	58	F	II	G2	3.5	Wild		
HCV60T	69	M	IIIA	G2	7	Mutant	244	GGC→GCC (Gly→Ala)
HCV62T	74	F	I	G2	2.9	Wild		
HCV86T	73	F	I	G1	1.2	Wild		
HCV87T	74	M	stage II	G1	2.9	Wild		

G1, well differentiated HCC; G2, moderately differentiated HCC; G3, poorly differentiated HCC.

GCCTCATTCAGCTCTC-3′ (exon 10) as reported by Sjogren et al. [14]. The sequence of the PCR products was determined by dye terminator-based DNA sequencing as described elsewhere.

2.4. Immunohistologic evaluation of p53 nuclear accumulation

We used immunohistochemistry to evaluate the nuclear accumulation of p53 in five wt-p53 tumors and five mt-p53 tumors of the 22 HCC samples subjected to oligonucleotide array analysis. Sections of 4 μm thick were deparaffinized in xylene and progressively rehydrated in decreasing concentrations of alcohol. Subsequently, the sections were treated with the EnVision™+ System (Dako, Kyoto, Japan) and DO-7 antibody (Novacastra Laboratories, Tokyo, Japan) for p53 staining as described previously [15]. We considered specimens to be positive for nuclear staining when more than 10% of the p53 reactivity was localized within nuclei. Two investigators judged the samples independently with no other knowledge of the cases, and reproducibility was confirmed.

2.5. Gene expression analysis by high-density oligonucleotide array

We analyzed the levels of expression of approximately 6000 genes by high-density oligonucleotide array (HuGeneFL Array, Affymetrix, Santa Clara, CA, USA) as described previously [9–11]. Raw data for 15 of the 22 samples are available at http://surgery2.med.yamaguchi-u.ac.jp/research/DNAchip/ [10].

2.6. Gene selection by supervised learning method

We performed gene selection by a supervised learning method combined with random permutation tests as described previously [9,11]. Briefly, we first selected genes that showed an average difference of >20 arbitrary units (according to Affymetrix) in all 22 HCV-related HCC samples. Among approximately 6000 genes, this filtering resulted in the selection of 2168 genes. Among the selected genes, we used the Fisher ratio (Fisher criterion) to evaluate gene expression differences between wt-p53 tumors and mt-p53 tumors and ranked the selected genes in order of decreasing magnitude of Fisher ratio [9,11]. To decide how many genes should be considered, we used a random permutation test as described previously [9,11]. The random permutation of sample labels was repeated 1000 times. The Fisher ratios generated from the actual data were then assigned P values on the basis of the distribution of Fisher ratios from randomized data. From the distribution of these Fisher ratios, the 83 genes with Fisher ratios of >2.12 were determined to have statistically significant ($P<0.01$) differences in expression between wt-p53 tumors and mt-p53 tumors (Tables 2 and 3).

2.7. Identification of consensus p53 binding sequence by computer

It is generally accepted that wt-p53-induced genes contain a consensus binding site consisting of two copies of a 10-bp motif (5′-PuPuPuC(A/T)(T/A)GPyPyPy-3′) separated by 0–13 bp, whereas wt-p53-repressed genes do not [16,17]. Our present study focused on exons 5–9 of the *p53* cDNA, which contain the DNA binding domain. Mutation of this region disrupts transcriptional activity of wt-p53. It is possible that genes upregulated in a wt-p53 tumor in our series are regulated directly by wt-p53 at the level of transcription. To clarify this possibility, we searched DNA sequences as follows. Nucleotide sequences (the first ~3000 bp upstream of the translation initiation site) of each gene were obtained from LocusLink (http://www.ncbi.nlm.nih.gov/LocusLink/) and by homology search. To screen for the consensus p53 binding site, a computer was programmed according to the method described by Wang et al. [18], in which no more than three mismatches with the canonical consensus sequence were permitted, and all decamers must contain a cytosine at position 4 and a guanine at position 7.

2.8. Reverse transcriptase (RT)-PCR analysis

To validate the microarray data, we selected two genes (*PTMA* and *NUMA1*) randomly from the 83 *p53*-related genes (Tables 2 and 3). We then performed RT-PCR analysis of five wt-p53 HCC samples, five mt-p53 HCC samples, and four non-cancerous liver control samples. RT and PCR were performed as described previously [9,11]. PCR was performed for 24 cycles for *β-actin*, 27 cycles for *PTMA*, and 30 cycles for *NUMA1*. The primers used in this study were as follows: *PTMA*, 5′-GGGGAGGAAGAGGAGGAGGAA-3′ (sense) and 5′-TGGTGGAGAGCGCGTGTCATC-3′ (antisense); *NUMA1*, 5′-GAAGCAGCCGGTGTCAGAGAG-3′ (sense) and 5′-ATCTCCCTCTTGGCCTGGTGG-3′ (antisense); and *β-actin*, 5′-CCAGAGCAAGAGAGGTAT-3′ (sense) and 5′-CTGTGGTGGTGAAGCTGTAG-3′ (antisense). The expected sizes were 318 bp, 378 bp, and 436 bp for *PTMA*, *NUMA1*, and *β-actin* genes, respectively. PCR products were separated by electrophoresis on 1.5% agarose gels and visualized under ultraviolet light after ethidium bromide staining (Fig. 3).

2.9. Statistical analysis

Fisher's exact test, Student's t-test and Mann–Whitney's U-test were used to analyze differences in clinicopathologic features between HCCs with mt-p53 and those with wt-p53.

Table 2
Twenty-five genes underexpressed in HCCs with mt-p53 compared with those with wt-p53

Fisher ratio	Accession number	Abbreviation	Locus	p53 binding site[a]	Function
3.85	X13973	RNH	11p15.5	−710 to −687, −697 to −675	Inhibition of angiogenesis
3.69	U05659	HSD17B3	9q22	−2673 to −2647	Metabolism
3.60	M97287	SATB1	3p23	−1324 to −1301	Immune response
3.29	X60592	TNFRSF5	20q12–q13.2	−1133 to −1106, −318 to −297, −318 to −287	Immune response/cell cycle arrest
3.15	M37457	ATP1A3	19q13.2	ND[b]	Molecular transport
3.11	L76224	GRIN2C	17q25	−927 to −894	Signal transduction
3.00	Y11897	CHIC1	Xq13–q21	absent	Unknown
2.98	M37245	CTLA4	2q33	−2742 to −2719, −2413 to −2387	Immune response
2.97	M60284	TACR2	10q11–q21	−2980 to −2958, −2793 to −2760, −871 to −842, −716 to −683	Signal transduction
2.78	X12433	ABHD2	15q26	−2833 to −2800, −2422 to −2398, −1433 to −1403	Unknown
2.78	Y08265	CHERP	19p13.1	−2275 to −2255	Neurogenesis
2.53	HG2238-HT2321	NUMA1	11q13	−2813 to −2793, −2803 to −2771	Apoptosis
2.51	Z14000	RING1	6p21.3	absent	Transcription
2.45	M37755	PSG1	19q13.2	absent	Immune response
2.44	X85785	FY	1q21–q22	absent	Signal transduction
2.37	L10338	SCN1B	19q13.1	−1580 to −1557	Molecular transport
2.36	U03642	AGTRL1	11q12	−2388 to −2364, −1987 to −1958, −727 to −700	Signal transduction
2.31	HG2566-HT4867	MAPT	17q21.1	−2874 to −2854, −1379 to −1350	Cell motility/microtubule assembly
2.28	L18983	PTPRN	2q35–q36.1	−585 to −563, −521 to −499	Miscellaneous
2.27	X54870	D12S2489E	12p13.2–p12.3	−2694 to −2664	Immune response
2.24	X99325	STK25	2q37.3	−2667 to −2643, −2667 to −2635	Apoptosis
2.24	D87465	KIAA0275	10pter–q25.3	−2137 to −2116	Unknown
2.17	X52943	ATF7	12q13	absent	Transcription
2.14	U24056	KCNJ4	22q13.1	ND[b]	Molecular transport
2.13	HG3162-HT3339	GTF2A1	14q31.1	−1470 to −1440	Transcription

Accession numbers were obtained from PubMed (http://www3.nibi.nlm.nih.gov/PubMed/) or TIGR (http://www.tigr.org/tdb/hgi/searching/reports.html) databases.
Abbreviations were used based on the data from LocusLink (http://www.nibi.nlm.nih.gov/LocusLink/).
[a]DNA sequence was searched for the first 3000 bp upstream of the translation initiation site. Number corresponds to the upstream nucleotide of the translation initiation site.
[b]Not determined.

3. Results

Missense mutations in exons 5–9 of the *p53* cDNA were detected in five of 22 tumors (23%), and *p53* was intact in the remaining 17 tumors (Fig. 1, Table 1). On the basis of this result, the five mutant tumors and the 17 wild-type tumors were classified as mt-p53 and wt-p53, respectively. Although limited in terms of the numbers of samples, there were no significant differences in clinicopathologic features between tumors with mt-p53 and those with wt-p53. All five mt-p53 tumors were positive for nuclear accumulation of p53. Four of five wt-p53 tumors tested were negative for nuclear accumulation of p53 (data not shown). Thus, genetic abnormality of *p53* was consistent with nuclear accumulation of p53 as detected by immunohistochemistry.

Our supervised learning method identified 83 genes associated with *p53* status from more than 6000 genes screened (Fig. 2, Tables 2 and 3). Of these 83 genes, 25 were underexpressed, and 58 were overexpressed in mt-p53 HCCs compared with wt-p53 HCCs. These genes have various biological functions (Tables 2 and 3); the underexpressed genes included apoptosis-related genes (*NUMA1*, *STK25*), immune response-related genes (*SATB1*, *TNFRSF5*, and *CTLA4*), signal transduction-related genes (*GRIN2C*, *TACR2*, *FY*, and *AGTRL1*), and transcription-related genes (*ATF7*, *CTF2A1*, and *RING1*). The overexpressed genes included oncogenesis-related genes (*RAB1A*, *TPM3*, and *ADM*), cell proliferation-related genes (*SSR1*, *PTMA*), metastasis-related genes (*LAMP1*, *CTSL*), and cell cycle-related genes (*CCNG2*, *BZAP45*). Our computer search identified the consensus p53 binding sequence in the 3-kb region upstream of the translation initiation site in 18 of the 25 underexpressed genes and in 41 of the 58 overexpressed genes (Tables 2 and 3).

To validate our microarray data, we analyzed the expression levels of *PTMA* and *NUMA1* genes in 10 representative HCC samples (five wt-p53 and 5 mt-p53) and four non-cancerous liver specimens by RT-PCR. Expression levels of *PTMA* were markedly higher in mt-p53 HCC than in wt-p53 HCC or non-cancerous liver (Fig. 3). Expression levels of *NUMA1* were repressed in mt-p53 HCC compared with non-cancerous liver, with the exception of one sample (HCV43T in Fig. 3). In contrast, expression levels of *NUMA1* were higher in all samples of wt-p53 HCC than in those of non-cancerous liver. Thus, expression patterns by microarray were consistent with those by RT-PCR.

4. Discussion

Patients with HCV-related HCC have various backgrounds and divergent clinical courses, resulting in much heterogeneity between tumor samples [11]. Unlike in vitro studies with cell lines, in analyzing transcriptomes in human cancer tissues, it is crucial to take interpatient heterogeneity into consideration. With this concept in mind, we used the Fisher ratio and the random permutation test [9,11] to identify molecular signatures related to p53 status.

Table 3
Fifty-eight genes overexpressed in HCCs with mt-p53 compared with those with wt-p53

Fisher ratio	Accession number	Abbreviation	Locus	p53 binding site[a]	Function
8.75	M28209	RAB1A	2p14	−1692 to −1662, −722 to −702	Oncogenesis/Signal transduction
6.75	U81006	TM9SF2	13q32.3	−212 to −183, −125 to −101	Endosomal protein
6.61	X81003	PPP1R11	6p21.3	−1572 to −1548	Hemochromatosis candidate gene
6.28	X76228	ATP6V1E1	22q11.1	absent	Molecular transport
6.25	U02680	PTK9	12p11.22	−1850 to −1823, −305 to −283	Signal transduction
5.54	D87446	RW1	2q11.1	−2949 to −2927, −889 to −867, −889 to −863, −889 to −862, −876–844	Unknown
5.12	HG2755-HT2862	PLS3	Xq24	absent	Signal transduction
4.23	M19311	CALM2	2p21	−401 to −377	Signal transduction
4.23	Z12830	SSR1	6p23	−2086 to −2064, −2073 to −2053, −2073 to −2048, −1259 to −1239	Cell proliferation
4.13	L24804	TEBP	12q12	−1189 to −1168	Signal transduction
3.97	U47414	CCNG2	4q13.3	−1713 to −1693	Cell cycle
3.83	X68194	SYPL	7q11.23	−467 to −448	Molecular transport
3.72	D87684	UBXD2	2q14.3	−2115 to −2096	Unknown
3.67	M23294	HEXB	5q13	−1619 to −1597, −1231 to −1203	Metabolism
3.66	X92098	RNP24	12q24.31	absent	Molecular transport
3.64	U62136	UBE2V2	8q11.1	absent	Proteolysis/peptidolysis
3.61	D14658	KIAA0102	11q13.3	absent	Unknown
3.50	U73824	EIF4G2	11p15	absent	Translational modification
3.47	AF002020	NPC1	18q11–q12	−2712 to −2682, −2711 to −2682	Molecular transport
3.47	U14603	PTP4A2	1p35	−1254 to −1235	Signal transduction
3.43	D13630	BZW1	2q33	−1530 to −1510	Cell cycle
3.40	U86782	POH1	2q24.3	−1478 to −1455	Proteolysis/peptidolysis
3.40	X90872	HSGP25L2G	5q35.3	absent	Molecular transport
3.32	M28713	DIA1	22q13.2-q13.31	absent	Energy pathway
3.16	L38961	ITM1	11q23.3	−2769 to −2747, −2395 to −2372, −2381 to −2358, −2116 to −2086, −2095 to −2063, −1367 to −1341	Transmembrane protein
3.09	M26708	PTMA	2q35–q36	−1738 to −1715, −1352 to −1320	Cell proliferation
3.07	U41766	ADAM9	8p11.21	−412 to −388	Cell motility/cell adhesion
3.03	HG2463-HT2559	CDC42	1p36.1	−2846 to −2826, −1531 to −1501	Miscellaneous
2.99	HG4297-HT4567	PC4	5p13.3	−1919 to −1898, −1011 to −984	Transcription
2.97	M33521	BAT3	6p21.3	−2150 to −2128	Stress response
2.91	Z48042	M11S1	11p13	−639 to −609	Plasma membrane
2.88	M36341	ARF4	3p21.2–p21.1	absent	Signal transduction/ metabolism
2.80	U93205	CLIC1	6p22.1–p21.2	−2130 to −2104	Molecular transport
2.76	HG3514-HT3708	TPM3	1q21.2	−2884 to −2863, −2808 to −2784, −2404 to −2382	Oncogenesis
2.73	M75715	ETF1	5q31.1	−2310 to −2291, −2300 to −2281, −1281 to −1260, −388 to −365	Translation
2.68	J03263	LAMP1	13q34	−1258 to −1239	Metastasis
2.67	D49489	P5	2p25.1	−1802 to −1779	Metabolism
2.66	M38591	S100A10	1q21	−2197 to −2177	Cell proliferation
2.61	U18009	VATI	17q21	absent	Molecular transport
2.60	X56468	YWHAQ	2p25.1	absent	Signal transduction/cell cycle
2.57	S65738	DSTN	20p11.23	−1971 to −1946, −1890 to −1869, −1562 to −1537	Cell structure
2.55	D14874	ADM	11p15.4	−1236 to −1204, −301 to −269	Oncogenesis
2.50	D80005	C9orf10	9q22.31	−606 to −580, −200 to −175	Unknown
2.43	Y00282	RPN2	20q12–q13.1	−647 to −628	Metabolism
2.40	D78577	YWHAH	22q12.3	−1637 to −1613, −1637 to −1612, −1588 to −1560, −1038 to −1017, −937 to −917, −937 to −906, −926 to −906	Signal transduction
2.38	M64992	PSMA1	11p15.1	−534 to −513	Proteolysis/peptidolysis
2.37	U02556	TCTE1L	Xp21	absent	Unknown
2.34	V00572	PGK1	Xq13	−1901 to −1877, −1886 to −1867	Signal transduction
2.34	AB000468	RNF4	4p16.3	−2876 to −2854, −2729 to −2704, −602 to −572	Transcription
2.30	X57346	YWHAB	20q13.1	−1127 to −1100	Signal transduction
2.27	U26312	CBX3	7p15.2	ND[b]	DNA binding protein
2.26	X74104	SSR2	1q21-q23	absent	Signal transduction
2.25	D00017	ANXA2	15q21–q22	−2356 to −2329	Cell proliferation
2.23	D86960	KIAA0205	1p36.13–q42.3	absent	Unknown
2.22	U82130	TSG101	11p15	−808 to −785, −498 to −466	Oncogenesis
2.18	X12451	CTSL	9q21–q22	absent	Proteolysis/peptidolysis

Table 3 (*Continued*).

Fisher ratio	Accession number	Abbreviation	Locus	p53 binding site[a]	Function
2.18	U62389	IDH1	2q33.3	absent	Energy pathway
2.14	J04611	G22P1	22q13.2–q13.31	−1513 to −1482	DNA repair

Accession numbers were obtained from PubMed (http://www3.nibi.nlm.nih.gov/PubMed/) or TIGR (http://www.tigr.org/tdb/hgi/searching/reports.html) databases.
Abbreviations were used based on the data from LocusLink (http://www.nibi.nlm.nih.gov/LocusLink/).
[a]DNA sequence was searched for the first 3000 bp upstream of the translation initiation site. Number corresponds to the upstream nucleotide of the translation initiation site.
[b]Not determined.

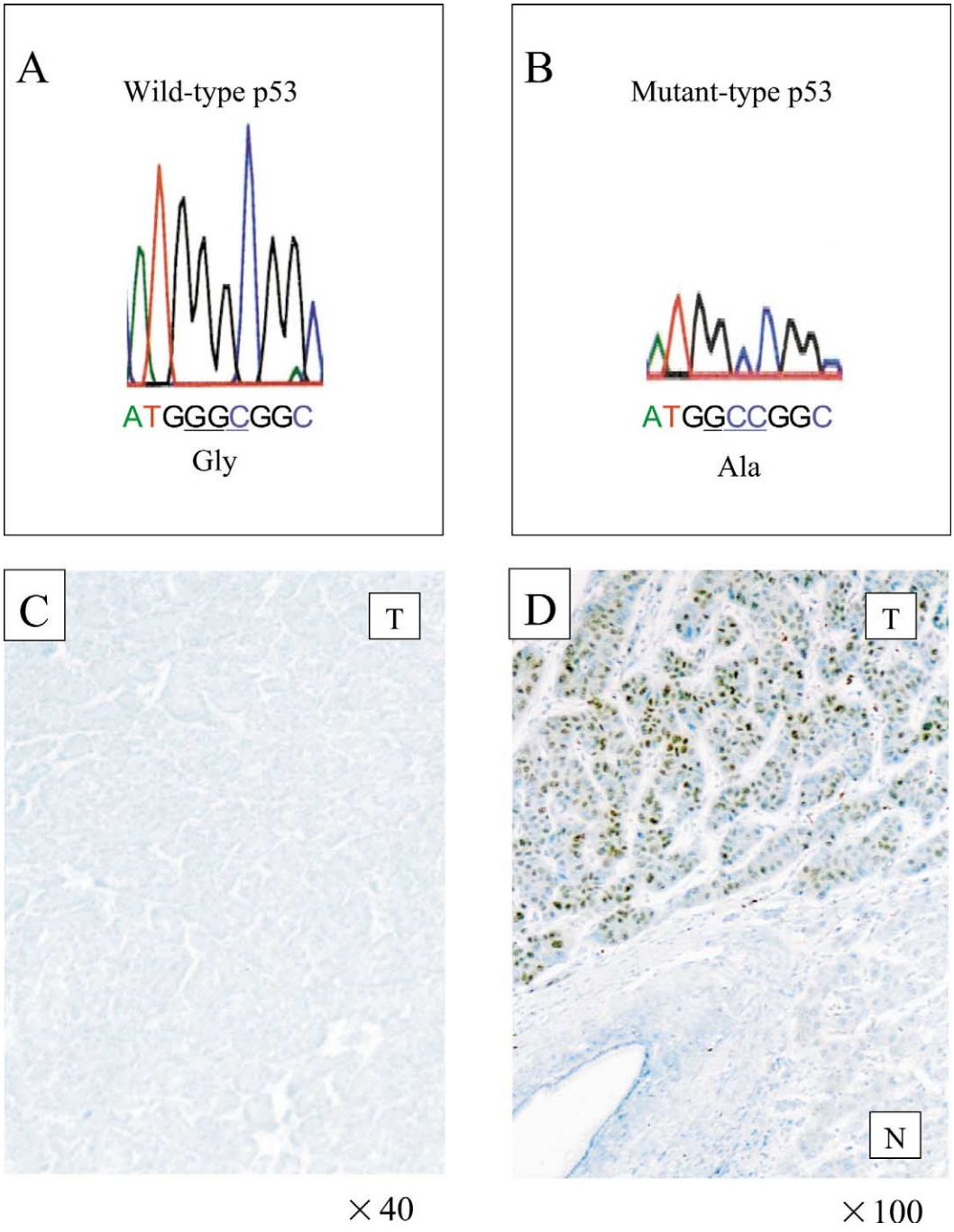

Fig. 1. *p53* status (A,B) and p53 immunostaining (C,D) in representative cases. A,B: Sequence chromatographs of codon 244 of HCV2T and HCV60T, respectively. HCV2T carries wild-type *p53*, whereas HCV60T shows a missense mutation of Gly (GGC) to Ala (GCC) at codon 244. C: Lack of p53 staining in HCV2T (40×). No cancer cells are stained. D: p53 staining in a tissue section from HCV60T (100×). Most of the cancer cell nuclei stain with the anti-p53 antibody (DO-7). Note that normal hepatocytes show no p53 staining. T, primary tumor; N, non-tumorous liver.

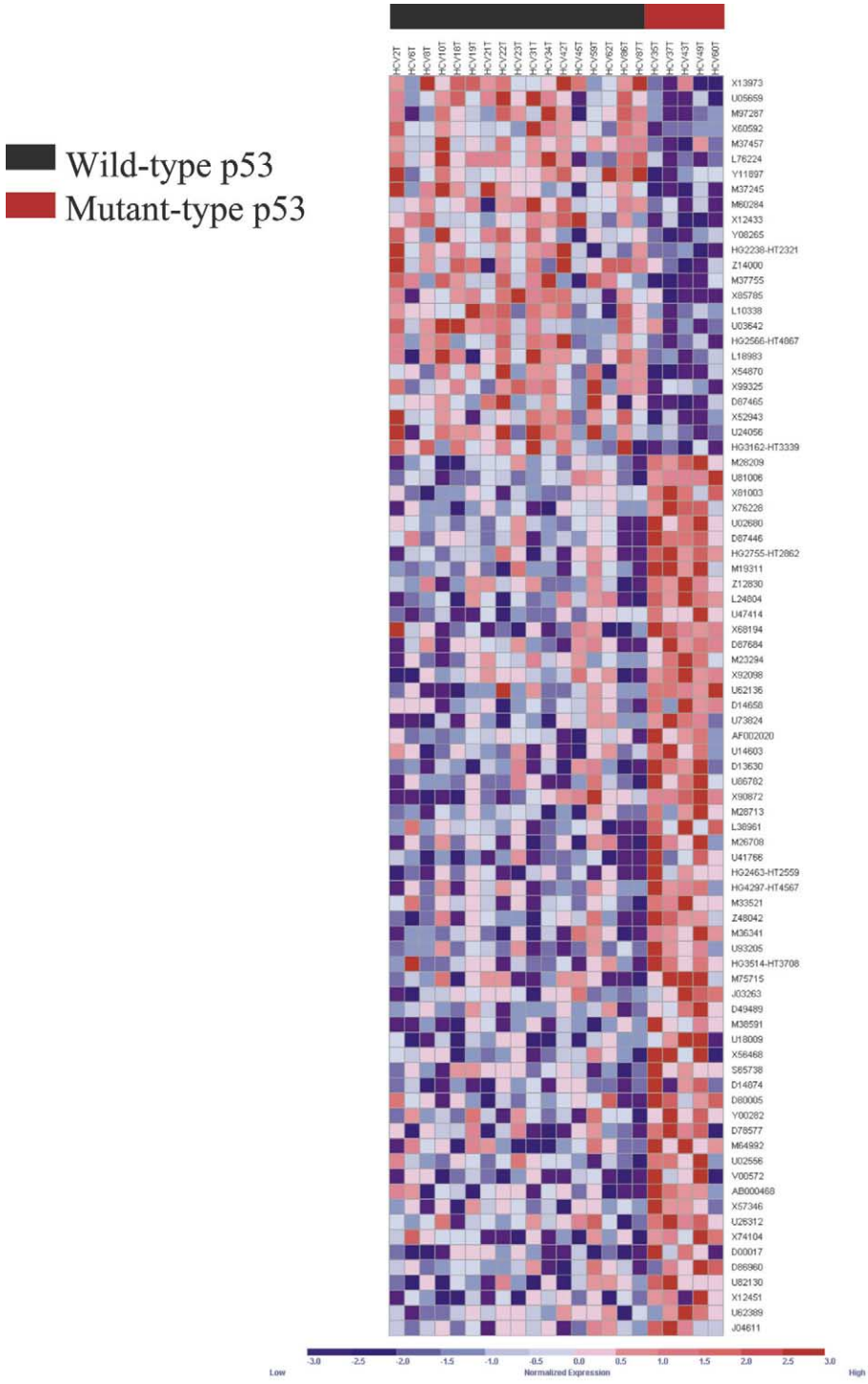

Fig. 2. Gene expression profiles linked to *p53* status in HCC. Samples are listed horizontally. Upper panel, 25 genes underexpressed in tumors with mt-p53 compared with wt-p53. Lower panel, 58 genes overexpressed in mt-p53 tumors compared to wt-p53 tumors. Genes are ranked in decreasing order of Fisher ratio (see Section 2) and listed according to accession number. The accession numbers were obtained from the PubMed (http://www3.ncbi.nlm.nih.gov/PubMed/) or TIGR (http://www.tigr.org/tdb/hgi/searching/reports.html) databases.

The most striking finding of our study is that HCV-infected HCCs with wt-p53 and those with mt-p53 differ significantly in their gene expression patterns. Our gene selection revealed that cell cycle-related genes (*CCNG2*, *BZAP45*) and cell proliferation-related genes (*SSR1*, *ANXA2*, *S100A10*, and *PTMA*) were overexpressed in mt-p53 tumors compared with wt-p53 tumors. On the basis of altered levels of these genes, it is reasonable to assume that mt-p53 tumors have higher malignant potentials than do wt-p53 tumors. This concept is supported by previous reports that p53 mutations constitute an unfavorable prognostic factor related to recurrence in HCC [19,20]. Our present results with *CCNG2* and *PTMA* are consistent with those of previous reports [21,22]. *CCNG2* encodes human cyclin G2. Bates et al. [21] reported that

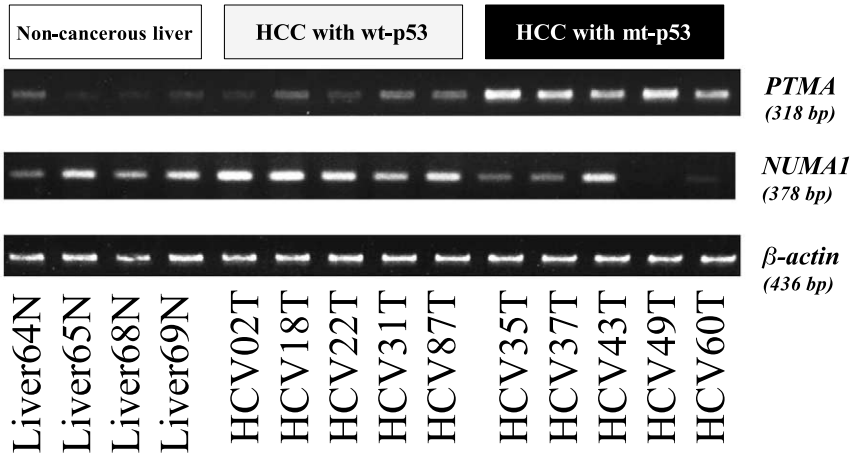

Fig. 3. RT-PCR of *PTMA* and *NUMA1* in representative cases. To validate the microarray data, we performed RT-PCR of five wt-p53 HCC samples (HCV02T, HCV18T, HCV22T, HCV31T, and HCV87T), five mt-p53 HCC samples (HCV35T, HCV37T, HCV43T, HCV49T, and HCV60T), and four non-cancerous liver samples (Liver64N, Liver65N, Liver68N, and Liver69N). *PTMA* mRNA was more abundant in mt-p53 HCCs than in wt-p53 tumors and non-cancerous livers, whereas *NUMA1* was repressed in four of five mt-p53 HCCs. The microarray data were validated by RT-PCR.

although induction of cyclin G1 expression is p53-dependent, activation of cyclin G2 expression by DNA-damaging agents occurs in the absence of p53. Transcription of *PTMA* is directly repressed by p53 [22], indicating that it is relatively overexpressed in mt-p53 tumors. Interestingly, it was reported that *PTMA* is overexpressed in HCC [23]. Our present finding that PTMA is predominantly expressed in mt-p53 HCC suggests that *PTMA* in mt-p53 HCC may be a molecular target for cancer therapy.

Unexpectedly, our computer search detected the consensus p53 binding sequence in the 3-kb region upstream of the translation initiation site in both overexpressed and underexpressed genes. This result suggests that in human cancer tissues the p53-associated transcription system possesses more complicated pathways. This concept is supported by our result that, with the exceptions of *CCNG2* and *PTMA*, most of the 83 genes listed here are distinct from those *p53*-related genes identified previously [22]. Additionally, in our in vivo study, it is unclear whether wt-p53 is activated in tumor tissues. Further studies are required to clarify whether these genes are directly regulated by wt-p53.

Many immune system-related genes, including *SATB1*, *TNFRSF5*, and *CTLA4*, were underexpressed in mt-p53 tumors. Because we did not perform laser capture microdissection (LCM), it is possible that the cancer samples tested contained stromal cells at concentrations of 5–10%. It is also important to consider that the expression patterns of the 83 genes identified here did not necessarily originate from the cancer cells themselves. Recently, we found that vimentin is produced preferentially by stromal cells within cancer tissues, and downregulation of vimentin expression is associated with early intrahepatic recurrence of HCC [10]. This is a clear example of identification of a gene derived from stromal cells, suggesting that gene analyses following LCM may fail to provide such an important finding. Mouta Carreira et al. observed the withdrawal of intratumor lymphatics in HCC tissue compared with non-tumorous liver tissue [24]. The decreased levels of immune system-related genes observed in mt-p53 HCCs in the present study may reflect the withdrawal of stromal cells within cancer tissues or downregulation of the immune system reaction against cancer cells in parallel with tumor progression. Further studies are needed to clarify whether these genes are derived from cancer cells or stromal cells and whether the consensus sequences identified here function as regulators of p53-dependent transcription.

Our present data provide unique evidence that *PTP4A2* is overexpressed and *ATF7* is underexpressed in mt-p53 tumors. *PTP4A2* (*PRL-1*) encodes a nuclear protein-tyrosine phosphatase that is an immediate-early gene in liver regeneration and is positively associated with growth, including fetal and neoplastic hepatic growth [25]. It is, therefore, reasonable to assume that upregulation of *PTP4A2* promotes cancer cell proliferation in mt-p53 HCC. Because a recent study revealed that *ATF7* interacts directly with *PTP4A2* [26], these two genes are likely to have biologic actions in response to p53 status. Our data also show that *annexin II* (*ANXA2*) and *S100A10*, which are involved in cell proliferation, are overexpressed in mt-p53 tumors. Interestingly, S100A10 is an annexin II ligand, suggesting that the two genes are closely related to the growth of HCV-related HCC with mt-p53. Thus, we expect that our data will aid in understanding the role of *p53* in the pathogenesis of HCV-related HCC.

In conclusion, our present data show that HCV-infected HCCs with wt-p53 and those with mt-p53 differ significantly in their gene expression patterns. Because *p53* has attracted a great deal of attention as a molecular target for cancer gene therapy [27], our present data may serve as a useful tool for predicting the efficacy of *p53*-based therapies.

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