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SNPs in the promoter region of the osteopontin gene as a marker predicting the efficacy of interferon-based therapies in patients with chronic hepatitis C

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Background. The T-helper (Th)1 immune reaction is essential for the eradication of hepatitis C virus (HCV) during interferon (IFN) therapy in patients with chronic hepatitis C. Osteopontin is a cytokine crucial for the initiation of the Th1 response. Recently, we identified four single-nucleotide polymorphisms (SNPs) in the promoter region of the osteopontin gene (*OPN*), at nucleotide (nt) -155, -443, -616, and -1748, and suggested that the SNP at nt -443 was a marker reflecting hepatitis activity in patients with HCV. Therefore, we examined the possibility that SNPs in *OPN* were also markers predicting the therapeutic efficacy of IFN in patients with chronic hepatitis C. ***Methods.*** Blood was collected from 77 patients with chronic hepatitis C who had received either IFN monotherapy or IFN-ribavirin combination therapy (IFN-based therapies). SNPs in *OPN*, *MxA*, *MBL*, and *LMP7* were analyzed by Invader assay. ***Results.*** Promoter SNPs of *OPN* at nt -155, -616, and -1748 showed linkage disequilibrium at 100% to each other. Sustained virological response (SVR) was observed in 58% of all patients. The SVR rate was higher in patients with the G/G or G/A alleles in the *OPN* promoter SNP at nt -1748 than in those with A/A (85% vs 45%; $P < 0.05$). The SVR rate was also higher in patients with T/T at nt -443 than in those with C/C or C/T (86% vs 47%; $P < 0.05$). Such differences were particularly evident in patients with HCV genotype 1b who had a pretreatment viral load greater than 100 KIU/ml. All the patients who had G/G or G/A at nt -1748 and T/T at nt -443 obtained an SVR. On the other hand, there was no relationship between the efficacy of IFN-based therapies and SNPs in *MxA*, *MBL*, and *LMP7*, which had been shown to have association with the response to IFN monotherapies. ***Conclusions.*** SNPs in the promoter region of *OPN* may be useful as a marker to predict the efficacy of IFN-based therapies in patients with chronic hepatitis C, and further investigation regarding their real significance is warranted in a large series of patients.

Key words: osteopontin, promoter SNPs, chronic hepatitis C, interferon

Introduction

Persistent infection with the hepatitis C virus (HCV) frequently provokes hepatocyte necrosis by cytotoxic T lymphocytes, due to the T-helper (Th)1 immune reaction,[1] and extracellular matrix deposition in the space of Disse,[2] leading to hepatic fibrosis by the conversion of the normal architecture into structurally abnormal nodules, and finally, to liver cirrhosis. The incidence of hepatocellular carcinoma increased with the degree of hepatic fibrosis in patients with chronic hepatitis C and the annual incidence was 7.9% in patients with liver cirrhosis.[3] Therefore, antiviral therapies, with interferon (IFN) alone or in combination with ribavirin, are required to reduce the risk of carcinogenesis in patients with chronic hepatitis C.

It is well known that the efficacy of IFN monotherapy or IFN-ribavirin combination therapy (IFN-based therapies) depends on the HCV genotype and serum HCV-RNA level; genotype 1b, the predominant genotype of HCV in Japan,[4] had poorer responsiveness to IFN-based therapies than genotypes 2a or 2b, and having a viral load of 100 KIU/ml or more, by Amplicor monitor assay (Roche Diagnostica, Tokyo, Japan) was also an indicator of poor responsiveness to IFN.[5] On the other hand, serum HCV-RNA level was shown to decrease with a biphasic kinetic pattern within 2 weeks after the initiation of IFN-based therapies.[6,7] Neumann

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Table 1. Therapies for chronic hepatitis C and genotype and serum level of HCV in patients

	Genotype 1b; <100 KIU/ml	Genotype 1b; ≥100 KIU/ml	Genotype 2a or 2b
(a) Pegylated-IFN-α2b + ribavirin	0[a]	6	0
(b) IFN-α2b + ribavirin	0	7	0
(c) IFN-α2b	1	5	4
(d) IFN-β	5	8	11
(e) IFN-β + IFN-alphacon	0	6	6
(f) IFN-β + IFN-α2b + ribavirin	1	10	7

The schedules for each therapy are outlined in the "Patients and methods" section
[a] Number of patients

et al.[6] suggested that the rapid decrease in the first phase, observed within 1 or 2 days, was due to the direct effect of IFN on HCV production or release from hepatocytes, while the slower decrease in the second phase resulted from immune-mediated elimination of hepatocytes infected with HCV.[6] Furthermore, it was shown that the slope of the decline in the second phase was high in all patients with a sustained virological response (SVR) and low in most non-responders to IFN therapies.[8] Thus, the efficacy of IFN-based therapies may be influenced not only by viral factors but also by host factors, such as the Th1 immune reaction.

Osteopontin is an extracellular matrix protein with an RGD motif, and it is physiologically expressed in the kidney and bone. Previously, we found that osteopontin was expressed in activated Kupffer cells and stellate cells, and that osteopontin contributed to the migration of macrophages into necrotic areas in injured rat liver.[9,10] On the other hand, it was shown that osteopontin also acted as a cytokine essential for the initiation of the Th1 immune response in mice,[11] and that genetic polymorphisms in the osteopontin gene (*OPN*) determined the magnitude of the immune reaction to bacterial infection in mice.[12] Recently, we found four single-nucleotide polymorphisms (SNPs) in the promoter region of human *OPN*, and suggested that SNP in *OPN* at nucleotide (nt) -443 affected hepatitis activity in patients with chronic hepatitis C.[13] From these observations, we assumed that SNPs in the promoter region of *OPN* might be markers to predict the efficacy of IFN-based therapies in HCV-infected patients.

In the present study, we analyzed SNPs in the promoter region of *OPN* in patients with chronic hepatitis C treated with IFN alone or with IFN plus ribavirin, and evaluated the significance of these SNPs as a marker predicting the efficacy of these therapies, as compared with SNPs in the myxovirus resistance protein A gene (*MxA*), the mannose-binding lectin gene (*MBL*), and the low-molecular-mass polypeptide 7 gene (*LMP7*), which had been reported to have associations with the response to IFN therapies.[14–17]

Patients and methods

Patients and interferon-based therapies

The patients were 77 Japanese with chronic hepatitis C who had medical examinations at the outpatient clinic of Saitama Medical School Hospital in August 2002, and who had finished IFN-based therapies by March 2004. All the patients were positive for HCV-RNA before the therapies and negative for hepatitis B virus surface antigen in the sera. The diagnosis of chronic hepatitis was made by histological findings in liver biopsy specimens and/or by serum biochemical tests and peripheral blood cell counts. Documented informed consent for gene analysis was obtained from all the patients.

Each patient received one of six schedules of therapy with IFN (Table 1), as follows; (a) subcutaneous injection of pegylated (Peg)-IFN-α2b, at 1.5 μg/kg body weight once a week, combined with daily oral administration of ribavirin at 600, 800, or 1000 mg/day for patients with a body weight of less than 60 kg, between 60 and 80 kg, and more than 80 kg, respectively, for 48 weeks; (b) intramuscular injection of IFN-α2b, at 6 MU daily for 2 weeks and three times a week for the following 46 weeks, combined with administration of ribavirin as in schedule (a) for 48 weeks; (c) injection of IFN-α2b at 10 MU daily for 4 weeks and three times a week for the following 20 weeks; (d) intravenous injection of IFN-β, at 3 MU, at 12-h intervals for 4 weeks and at 6 MU at 24-h intervals for the following 2 to 8 weeks; (e) injection of IFN-β at 3 MU at 12-h intervals for 4 weeks, and subcutaneous injection of IFN-alphacon at 18 MU three times a week for the following 24 weeks; or (f)

injection of IFN-β at 3 MU at 12-h intervals for 4 weeks and injection of IFN-α2b at 10 MU three times a week for the following 24 weeks, with administration of ribavirin at 600 mg or 800 mg for patients with body weights of less than 60 kg or 60 kg or more, respectively. The intervals between IFN-β injections, and the doses of IFN and ribavirin were changed when severe side effects occurred.

Analysis of serum HCV-RNA and determination of therapeutic efficacy of interferon alone or interferon plus ribavirin

The serum HCV-RNA level was measured using a polymerase chain reaction (PCR) Kit (Amplicor HCV Monitor; Roche Diagnostica, Tokyo, Japan). The HCV genotype was determined on the basis of the sequence of the core region, according to the method of Okamoto et al.[18]

Patients in whom serum HCV-RNA was not detected by PCR (Cobas Amplicor; Roche Diagnostica) for 6 months after the discontinuation of IFN-based therapies were classified as patients with an SVR, and the other patients were classified as those with non-response (NR).

Analysis of SNPs in the genes for osteopontin, myxovirus resistance protein A, mannose-binding lectin, and low-molecular-mass peptide 7

Genomic DNA was extracted from peripheral blood mononuclear cells. SNPs in the promoter region of *OPN*, at nt -155, -443, -616, and -1748; *MxA*, at nt -88 and -123; and *MBL*, at nt -221, and SNPs in *MBL* at G54D and *LMP7* at Q49K were determined by Invader assay.[19]

The Invader assay was done as described previously,[20] with minor modifications. Primer probes and the Invader oligonucleotides for each SNP (Tables 2 and 3) were designed with Invader Creator software (Third Wave Technologies, Madison, WI, USA) to have theoretical annealing temperatures of 63°C and 77°C, respectively. The reactions were performed using 384-well Invader assay fluorescence resonance energy transfer (FRET) detection plates (Third Wave Technologies), in which Cleavase XI enzyme, both F (FAM) dye and R (Redmond Red) dye (Epoch Biosciences, Redmond, WA, USA) FRET cassettes and reaction buffer were dried on each well. Three microliters of a mixture consisting of the appropriate primary probe, Invader oligonucleotide, and $MgCl_2$ was added to the wells, followed by the addition of 3 μl of the heat-denatured genomic DNA (≥10 ng/μl), and overlaid with 6 μl of mineral oil (Sigma Chemical, St. Louis, MO, USA). The plates were incubated at 63°C for 4 h in a DNA thermocycler (RTC-200; MJ Research, Watertown, MA, USA), and then kept at 4°C until fluorescence measurements were done. The fluorescence intensities were measured on a Cytoflour 4000 fluorescence plate reader (Applied Biosystems, Foster City, CA, USA) with excitation at 485/20 nm (wavelength/bandwidth) and emission at 530/25 nm for F dye detection, and excitation at 560/20 nm and emission at 620/40 nm for R dye detection.

Statistical analysis

The proportions of patients with an SVR were compared among patients with different alleles of the SNPs by Fisher's exact test. Also, these proportions were compared in patients with different backgrounds for viral markers. *P* values of less than 0.05 were regarded as statistically significant.

Table 2. Probes for Invader assay of SNPs in the promoter region of *OPN*

SNP	Type of Probe	Probe sequences
nt -155	Primary probe 1	acggacgcggagCCAAAAACGCACACAC
	Primary probe 2	cgcgccgaggCAAAAACGCACACACAC
	Invader probe	CCACACTTCCCCCTCTGGTTTTGTGGTTAAAACAAAAAAAAAT
nt -443	Primary probe 1	cgcgccgaggAAACTTGCCTCTGTCC
	Primary probe 2	acggacgcggagGAACTTGCCTCTGTCC
	Invader probe	GAAGGCTATTGTTCAAGCCTGCAAGGAGTTCAGAT
nt -616	Primary probe 1	acggacgcggagAAGGATGACTGCTTGAG
	Primary probe 2	cgcgccgaggCAGGATGACTGCTTGAG
	Inavder probe	GATGTTGCAGAAGTAAAGCAGTTTCTGACTGAGAGT
nt -1748	Primary probe 1	acggacgcggagGGACTTCCCTCCACTAA
	Primary probe 2	cgcgccgaggAGACTTCCCTCCACTAAA
	Invader probe	GGCACAGAGTAAACTACAGTAAATCCTGTGGAAATTTTGTTGTTTTTAGAATTTTCT

Lower-case letters indicate the flap sequences of primary probes
nt, nucleotide

Table 3. Probes for Invader assay of SNPs in the genes for Myxovirus resistance protein A (*MxA*), mannose-binding lectin (*MBL*), and low-molecular-mass peptide 7 (*LMP7*)

SNP	Type of probe	Probe sequences
MxA nt -88	Primary probe 1	acggacgcgagGCCCGGAGCCG
	Primary probe 2	cgcgccgaggTCCCGGAGCCGC
	Invader probe	GGGGCCAGGAGCTAGGTTTCGTTTCTGCC
MxA nt -123	Primary probe 1	acggacgcggagGCAGCACTTGCCTC
	Primary probe 2	cgcgccgaggTCAGCACTTGCCTCG
	Invader probe	CCTAGCTCCTGGCCCCGCACCTC
MBL nt -221	Primary probe 1	acggacgcggagGGAAAGCATGTTTATAGTCTTC
	Primary probe 2	cgcgccgaggCGAAAGCATGTTTATAGTCTTC
	Invader probe	GCACGGTCCCATTTGTTCTCACTGCCACT
MBL G54D	Primary probe 1	acggacgcggagGCACCAAGGGAGAAAAG
	Primary probe 2	cgcgccgaggACACCAAGGGAGAAAAG
	Invader probe	GCTTCCCAGGCAAAGATGGGCGTGATGT
LMP7 Q49K	Primary probe 1	acggacgcggagCAGGTCGGGGCAG
	Primary probe 2	cgcgccgaggAAGGTCGGGGCAG
	Invader probe	CCAGAGCTCGCTTTACCCCGGGGAATGT

Lower-case letters indicate the flap sequences of primary probes

Table 4. SNPs in the genes for osteopontin (*OPN*), Myxovirus resistance protein A (*MxA*), mannose-binding lectin (*MBL*), and low-molecular-mass polypeptide 7 (*LMP7*) in patients with chronic hepatitis C treated with interferon with or without ribavirin

SNP	Number of patients (%)		
OPN nt -155	G/G Homozygotes 1 (1.3)	G/- Heterozygotes 25 (32.5)	–/– Homozygotes[a] 51 (66.2)
OPN nt -443	C/C Homozygotes 15 (19.5)	C/T Heterozygotes 40 (51.9)	T/T Homozygotes 22 (28.6)
OPN nt -616	T/T Homozygotes 1 (1.3)	T/G Heterozygotes 25 (32.5)	G/G Homozygotes 51 (66.2)
OPN nt -1748	G/G Homozygotes 1 (1.3)	G/A Heterozygotes 25 (32.5)	A/A Homozygotes 51 (66.2)
MxA nt -88	G/G Homozygotes 36 (46.8)	G/T Heterozygotes 34 (44.2)	T/T Homozygotes 7 (9.1)
MxA nt -123	C/C Homozygotes 29 (37.7)	C/A Heterozygotes 44 (57.1)	A/A Homozygotes 4 (5.2)
MBL nt -221	G/G Homozygotes 67 (87.0)	G/C Heterozygotes 10 (13.0)	C/C Homozygotes 0
MBL G54D	G/G Homozygotes 57 (74.0)	G/A Heterozygotes 13 (16.9)	A/A Homozygotes 7 (9.1)
LMP7 Q49K	C/C Homozygotes 57 (74.0)	C/A Heterozygotes 19 (24.7)	A/A Homozygotes 1 (1.3)

[a] Deletion mutation

Results

Demographic and clinical features of the patients, and therapeutic efficacy of interferon alone or interferon plus ribavirin

The patients were 45 men and 32 women, aged 53.3 ± 10.0 years (mean ± SD), with a range of 25 to 70 years. The genotype of serum HCV-RNA was 1b in 49 patients (63.6%), 2a in 23 (29.9%), and 2b in 5 (6.5%). Of the 49 patients with HCV genotype 1b, 7 patients (14.3%) had a pretreatment viral load of less than 100 KIU/ml (patients with genotype 1b and low titer) and 42 patients (85.7%) had a pretreatment viral load of 100 KIU/ml or more (patients with genotype 1b and high titer).

SVR was achieved in 45 patients (58.4%). The SVR rate was significantly higher in patients with genotype

Table 5. SNPs in the promoter region of *OPN* and response to interferon-based therapies

SNP	Total			Genotype 1b; <100 KIU/ml			Genotype 1b; ≥100 KIU/ml			Genotype 2a or 2b		
nt -155	G/G	G/–	–/–[c]	G/G	G/–	–/–	G/G	G/–	–/–	G/G	G/–	–/–
SVR[a]	1	21	23	0	3	3	1	8	8	0	10	12
NR[b]	0	4	28	0	0	1	0	3	22	0	1	5
P values[d]												
G/G and G/– vs –/–		0.018			>0.999			0.006			0.355	
nt -443	C/C	C/T	T/T	C/C	C/T	T/T	C/C	C/T	T/T	C/C	C/T	T/T
SVR	7	19	19	1	4	1	4	5	8	2	10	10
NR	8	21	3	1	0	0	7	15	3	0	6	0
P values												
C/C and C/T vs T/T		0.002			>0.999			0.029			0.062	
nt -616	T/T	T/G	G/G	T/T	T/G	G/G	T/T	T/G	G/G	T/T	T/G	G/G
SVR	1	21	23	0	3	3	1	8	8	0	10	12
NR	0	4	28	0	0	1	0	3	22	0	1	5
P values												
T/T and T/G vs G/G		0.018			>0.999			0.006			0.355	
nt -1748	G/G	G/A	A/A	G/G	G/A	A/A	G/G	G/A	A/A	G/G	G/A	A/A
SVR	1	21	23	0	3	3	1	8	8	0	10	12
NR	0	4	28	0	0	1	0	3	22	0	1	5
P values												
G/G and G/A vs A/A		0.018			>0.999			0.006			0.355	

[a] SVR, Sustained virological response
[b] NR, Virological non-response
[c] Deletion mutation
[b] *P* values by Fisher's exact test

1b and a low titer than in those with genotype 1b and a high titer (85.7% vs 40.5%, respectively; $P = 0.041$). Twenty-two patients with genotype 2a or 2b obtained an SVR (78.6%).

SNPs in the genes for osteopontin, myxovirus resistance protein A, mannose-binding lectin and low-molecular-mass peptide 7

As shown in Table 4, of the four SNPs in the promoter region of *OPN*, SNPs at nt -155, -616, and -1748 showed linkage disequilibrium at 100% to each others. On the other hand, there was no relationship between the prevalence of the four SNPs in *OPN* and each SNP in the *MxA*, *MBL*, and *LMP7* genes.

Table 5 shows that the response to IFN-based therapies differed depending on the alleles the three SNPs with 100% linkage disequilibrium and the SNP at nt -443. In regard to the SNP at nt -1748, the SVR rate was 84.6% (22/26) in patients with the G/G or G/A alleles, and this was significantly higher than the rate in those with the A/A allele (45.1%; 23/51). The SVR rate in patients with the T/T allele in the SNP at nt -443 (86.4%; 19/22) was also significantly higher than the SVR rate in those with the C/C or C/T alleles (47.3%; 26/55). Similar results were found in patients with genotype 1b and a high titer. Furthermore, all 14 patients showing G/G or G/A in the SNP at nt -1748 and T/T in the SNP at nt -443 obtained an SVR after IFN-based therapies.

As shown in Table 6, there was no relationship between the SVR rate and SNPs in the *MxA*, *MBL*, and *LMP7* genes, except that the rate in patients with G/G or T/T in the promoter SNP of *MxA* at nt -88 tended to be higher than the rate in those with G/T in the group of patients with genotype 1b and a high titer.

Discussion

The present study was designed to evaluate the usefulness of SNPs in the promoter region of *OPN* at nt -155, -443, -616, and -1748 as a marker to predict the therapeutic efficacy of IFN alone or combined with ribavirin in patients with chronic hepatitis C. Alleles of SNPs were determined by the Invader assay, an assay that does not include DNA amplification by polymerase chain reaction (PCR), and which is applicable to the measurement of many samples.[19] Among the four SNPs

Table 6. SNPs in the genes for myxovirus resistance protein A (*MxA*), mannose-binding lectin (*MBL*), and low-molecular-mass polypeptide 7 (*LMP7*) and response to interferon-based therapies

SNP	Total			Genotype 1b; <100 KIU/ml			Genotype 1b; ≥100 KIU/ml			Genotype 2a or 2b		
MxA At nt -88	G/G	G/T	T/T	G/G	G/T	T/T	G/G	G/T	T/T	G/G	G/T	T/T
SVR[a]	22	19	4	3	3	0	10	4	3	9	12	1
NR[b]	14	15	3	1	0	0	8	14	3	5	1	0
P values[c]												
G/T vs C/G and T/T		0.816			>0.999			0.057			0.173	
MxA At nt -123	C/C	C/A	A/A	C/C	C/A	A/A	C/C	C/A	A/A	C/C	C/A	A/A
SVR	26	16	3	3	3	0	10	5	2	13	8	1
NR	18	13	1	1	0	0	12	12	1	5	1	0
P values												
C/C vs C/A and A/A		>0.999			>0.999			0.543			0.375	
MBL At nt -221	G/G	G/C	C/C	G/G	G/C	C/C	G/G	G/C	C/C	G/G	G/C	C/C
SVR	38	7	0	6	0	0	14	3	0	18	4	0
NR	29	3	0	1	0	0	22	3	0	6	0	0
P values												
G/G vs G/C		0.514			>0.999			0.672			0.549	
MBL G54D	G/G	G/A	A/A	G/G	G/A	A/A	G/G	G/A	A/A	G/G	G/A	A/A
SVR	33	8	4	5	1	0	11	4	2	17	3	2
NR	25	5	3	1	0	0	18	4	3	5	1	0
P values												
G/G vs G/C and A/A		>0.999			>0.999			0.738			>0.999	
LMP7 Q49K	C/C	C/A	A/A	C/C	C/A	A/A	C/C	C/A	A/A	C/C	C/A	A/A
SVR	33	11	1	4	2	0	13	3	1	16	6	0
NR	24	8	0	1	0	0	18	7	0	5	1	0
P values												
C/C vs C/A and A/A		>0.999			>0.999			>0.999			>0.999	

[a] SVR, Sustained virological response
[b] NR, Virological non-response
[c] *P* values by Fisher's exact test

described here, those at nt -155, -616, and -1748 had already been registered in a database of Japanese single-nucleotide polymorphisms (JSNP) and/or in the dbSNP (National Center for Biotechnology Information),[21] and we previously found that these SNPs showed linkage disequilibrium, with coefficients (D' and r^2) greater than 0.937 to each other in patients with chronic hepatitis C without any experience of IFN-based therapies.[13] The SNP at nt -443 was recently identified by our group,[13] and we found that the SNP at -443, but not the other three SNPs, had a close association with hepatitis activity in patients with chronic hepatitis C.[13] In the present study, the prevalence of the four SNPs of *OPN* (Table 4) was similar to that observed in patients without experience of IFN-based therapies,[13] and the SNPs at nt -155, -616, and -1748 showed 100% linkage disequilibrium to each other. These results suggested that there were no differences in genetic background, regarding *OPN*, between the patients with chronic hepatitis C who received IFN-based therapies and those without such experience at our hospital.

As shown in Table 5, the SVR rate differed depending on the alleles of the four SNPs in the promoter region of *OPN*. Such differences were particularly evident in patients with genotype 1b and a high titer. Moreover, all the patients with G/G or G/A at nt -1748 and T/T at nt -443 obtained an SVR after IFN-based therapies. Therefore, it was suggested that the SNP in the promoter region of *OPN* at nt -443 and the three SNPs at nt -155, -616, and -1748 with linkage disequilibrium were useful as a marker to predict the therapeutic efficacy of IFN alone or IFN plus ribavirin, especially in patients with genotype 1b and a high titer.

In this study, the SVR rate in patients with T/T in SNP of *OPN* at nt -443 was 86%. Previously, we reported that the frequency of T/T at nt -443 was about 3.5 times higher in patients with chronic hepatitis C with serum alanine aminotransferase (ALT) levels higher than 80 IU/l than in those with an ALT level lower than

30 IU/l.[13] The SNP at nt -443 is located 13 base pairs (bp) upstream of the cis-acting enhancing element of human *OPN*.[22] Considering that the Th1 response is involved in the development of inflammation in chronic hepatitis C[1] and that hepatocytes infected with HCV are eradicated by Th1 response during IFN-based therapies,[6] the SNP in *OPN* at nt -443 may be crucial in provoking diverse Th1 immune reactions against HCV through the regulation of osteopontin expression in the liver. This matter should be investigated in future by carrying out promoter assays with each allele at nt -443.

Hijikata et al.[14,15] reported that G/G at nt -88 and C/C at nt -123 in the promoter region of *MxA* were observed more frequently in patients with NR than in those with an SVR after IFN therapy. *MxA* was shown to encode an IFN-inducible protein that inhibited the replication of single-stranded RNA viruses.[23,24] Matsushita et al.[16] showed that the frequencies of C/C at nt -221 in the promoter region of *MBL* and A/A at G54D in *MBL* were higher in patients with NR than in those with an SVR. Mannose-binding lectin is an acute-phase reactant protein inducing the phagocytosis of macrophages through binding to the surface of pathogens, and it is known to be essential for the innate immune system.[25,26] Sugimoto et al.[17] found that the frequency of C/A at Q49K in *LMP7* was higher in patients with an SVR than in those with NR, especially in patients with serum HCV-RNA levels less than 100 KIU/ml. Low-molecular-mass polypeptides were shown to play a crucial role in human leukocyte antigen (HLA) class I-restricted antigen-presenting systems.[27] However, we found no relationship between the SVR rate after IFN-based therapies and the alleles of these SNPs in patients with chronic hepatitis C. As outlined in Table 1 and the "Patients and methods" section, most patients received IFN-ribavirin combination therapy or IFN monotherapy with 3 MU of IFN-β injection given at 12-h intervals for 4 weeks as an induction therapy. This monotherapy was shown to be superior in HCV antiviral effects to 6 MU IFN-β injection given at 24-h intervals.[28] IFN-ribavirin combination therapy was also reported to show a higher SVR rate than IFN monotherapy.[29] The rate of SVR in patients with genotype 1b and a high titer in the present study was 40.5%. In previous studies regarding SNPs in *MxA*, *MBL*, and *LKP7*, all the patients received IFN-α for 24 weeks or less.[14–17] After IFN-α therapy for 24 weeks, the SVR rate was reported to be only 7%–8% in patients with genotype 1b and a high titer.[30] The differences in the antiviral effects of these therapies may produce discrepancies in the results regarding SNPs in *MxA*, *MBL*, and *LMP7*. This matter should be further investigated in a large series of patients in whom standardized therapy with Peg-IFN-α2b and ribavirin is done for 48 weeks.

In conclusion, four SNPs in the promoter region of *OPN* may be useful as a marker to predict the efficacy of IFN-based therapies in patients with chronic hepatitis C.

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