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Hepatic expression of MxA and OAS1 in an *ex vivo* liver slice assay independently predicts treatment outcomes in chronic hepatitis C

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SUMMARY. Antiviral effect of interferon is mediated by the expression of interferon-stimulated genes (ISGs). However, because of the difficulty in obtaining paired liver biopsies before and after interferon treatment, the key ISGs expressed in human hepatocytes and responsible for interferon-based antiviral activities in chronic hepatitis C remain illusive. Prior to a standard course of peginterferon plus ribavirin therapy, 104 patients underwent a liver biopsy. A small piece of the liver biopsy sample from each patient was submitted for *ex vivo* tissue culture in the presence or absence of interferon. Hepatic expression of 8 ISGs was detected by RT-PCR. The ISG expression patterns and clinicopathological variables were correlated with subsequent treatment outcomes. Multivariate logistic regression analysis showed that hepatic MxA expression ($P = 0.008$) and leucocyte count ($P = 0.040$) independently predicted the end of therapeutic virological response, while hepatic OAS1 expression ($P = 0.003$), genotype 1 ($P = 0.002$), HCV-RNA level ($P = 0.007$), AST/ALT ratio ($P = 0.004$) and leucocyte count ($P = 0.034$) independently predicted the sustained virological response. Immunohistochemistry analysis showed that interferon-induced OAS1 expression localized to the hepatocytes. In conclusion, hepatic MxA and OAS1 expression predicted, respectively, the end of therapeutic and sustained virological responses in interferon-based treatment of chronic hepatitis C.

Key words: End of therapy response, interferon-stimulated gene, sustained virological response.

INTRODUCTION

Hepatitis C virus (HCV) infection is a global health problem affecting over 170 million people worldwide [1]. Chronic hepatitis C could lead to severe sequels such as liver cirrhosis and hepatocellular carcinoma. [2] HCV was first identified by the isolation of a cDNA clone derived from a non-A, non-B post-transfusion hepatitis patient in 1989 [3]. The standard treatment for chronic hepatitis C is the use of pegylated interferon in combination with ribavirin [4]. The goal of therapy is to achieve virological clearance at the end of treatment (end of treatment response) followed by a sustained virological clearance 6 months after the therapy is stopped (sustained virological response, SVR). Other direct antiviral agents are under development. Combination of these agents with the standard therapy improves the SVR [4].

Owing to frequent occurrence of prominent side effects, the risks and benefits of interferon and ribavirin treatment have to be carefully weighted [5]. Several factors are known to affect the outcome of antiviral therapy. HCV genotype is an important predictor and influences the choice of therapeutic durations [4,6]. Other factors associated with treatment outcome include HCV-RNA level, fibrosis stage, old age, men, African American, obesity, alcohol intake, insulin resistance, liver steatosis, interleukin-8 and 10 levels, TGF-beta level and IP-10 level [7–10].

The interferon family comprises several members [11]. Type I interferons include 13 subtypes of interferon alpha and 1 interferon beta. After RNA viruses infection, type I interferons are produced through the Toll-like receptor 3 mediated and the cytosolic RIG-I like helicase-mediated pathways. Subsequently, type I interferons bind to the interferon alpha receptor to activate intracellular signalling through the Jak-STAT

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pathway. As a result, hundreds of interferon-stimulated genes (ISGs) are generated through transcriptional activation, conferring antiviral activities. Among these ISGs, interferon-inducible double-stranded RNA-dependent protein kinase R (PRKR), 2′,5′ oligoadenylate synthetase 1 (OAS1) and Myxovirus resistance 1, interferon-inducible protein 78 (MxA) proteins are the best studied [12]. PRKR phosphorylates eukaryotic initiation factor, eIF2α, to inhibit translation of viral and cellular proteins [13]; OAS1 activates RNAse L-associated pathway, leading to cleavage of viral and cellular RNAs [14]; and Mx proteins perturb replication of negative-stranded RNA viruses [15]. Despite the established knowledge of antiviral functions of ISGs, the exact mechanisms by which therapeutic interferon exerts its effects against HCV remained illusive.

Several attempts have been made to establish the link between ISGs production and the treatment outcomes. However, these studies were either conducted by evaluating ISGs production in peripheral blood mononuclear cells or in pretreatment liver biopsies [16,17]. Paired liver biopsies were performed in only one study with limited number of patients [18].

Recently, an *ex vivo* liver slice culture system was established in our Research Centre [19,20]. In this study, we took advantage of this *ex vivo* system to evaluate whether ISG responses could be demonstrated in the cultured liver slices and whether the presence of any ISG response predicted the subsequent therapeutic outcomes. The ISGs tested in this study were selected according to a previous study [21].

MATERIALS AND METHODS

Patients

This study was approved by Institutional Review Board, Chang Gung Medical Centre. Under informed consent, 104 consecutive patients from October 2008 to April 2009, who received liver biopsy prior to a standard course of antiviral therapy prescribed at Chang Gung Medical Centre were evaluated. All of the patients were positive for antibody against HCV (anti-HCV), negative for hepatitis B virus surface antigen (HBsAg) and negative for human immunodeficiency virus. Gender, age, pretherapeutic aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, body mass index (BMI), total bilirubin, alpha-fetoprotein (AFP), albumin, leucocyte counts, polymorphonuclear cell counts, serum HCV-RNA levels, genotypes, Knodell histologic activity index (HAI), periportal scores, intralobular scores, portal inflammation scores, fibrosis scores and histologic appearance of fatty metamorphosis were documented (Table 1). Peginterferon alfa-2a (PEGASYS) 180 μg was given per week for 48 and 24 weeks for patients with genotype 1 and non-1, respectively. Ribavirin 1200 mg (1000 mg for genotype 2/3 patients) was given daily for the first 4 weeks. Thereafter, the dosage was adjusted individually according to haemoglobin concentrations (from 800 to 1200 mg per day). Patients were followed for at least 24 weeks after the end of treatment.

Table 1 Basic clinical data of patients included

Parameters	Value
Sample size, number of patients	104
Sex, Male/Female	48/56
Age, years	51.4 ± 11.8
Body mass index	24.8 ± 5.4
Biochemistries	
AST, U/L	100.4 ± 61.3
ALT, U/L	156.9 ± 109.0
Bilirubin, mg/dL	0.9 ± 0.4
Alpha-fetoprotein, ng/mL	13.1 ± 16.5
Albumin, g/dL	4.4 ± 0.6
White blood cells	
Neutrophils, $\times 10^3$	2.8 ± 1.1
Mononuclear cells, $\times 10^3$	2.7 ± 0.9
HCV-RNA, MIU/mL	0.69 ± 1.03
HCV genotype, 1/non-1*	54/50
Histology activity index	8 (3–14)
Periportal score	1 (0–4)
Lobular score	1 (0–3)
Portal inflammation score	3 (1–4)
Fibrosis score	3 (1–4)
Fatty liver, Yes/No	39/65

*Including genotype 2 and 3 in this study.

Anti-HCV was assayed using enzyme immunoassay kit (HCV-II; Abbott Laboratories, North Chicago, IL, USA). HBsAg was assayed using radioimmunoassay kits (Ausria-II RIA; Abbott Laboratories). HCV-RNA was quantified using COBAS AmpliPrep/COBAS TaqMan HCV test (Roche Diagnostics, Branchburg, NJ, USA) with a detection limit of 15 IU/mL. HCV genotypes were determined using Inno-Lipa HCV-II (Innogenetics, Zwijndrecht, Belgium).

Ex vivo *liver slice culture assay*

Biopsy was performed using the Bard Biopty-Cut biopsy needle (C.R. Bard, Covington, GA, USA) with a diameter of 1.2 mm (18 Gauge). Liver biopsy tissue core with a length greater than 2.0 cm was obtained from each patient. Two equal-sized slices of liver tissues, each measuring 1 mm in thickness, were separated from the main portion and immediately sent for liver slice culture experiment. Interferon was added to the culture medium of one of the liver slices (Fig. 1). The remaining (main) portions of biopsy tissues were submitted for routine pathology study.

The liver slice assay was performed according to our previous publication with some modifications [19,20]. Briefly, Vero cells were cultured in a 24-well culture plate with 2 mL minimal essential medium containing 20% foetal

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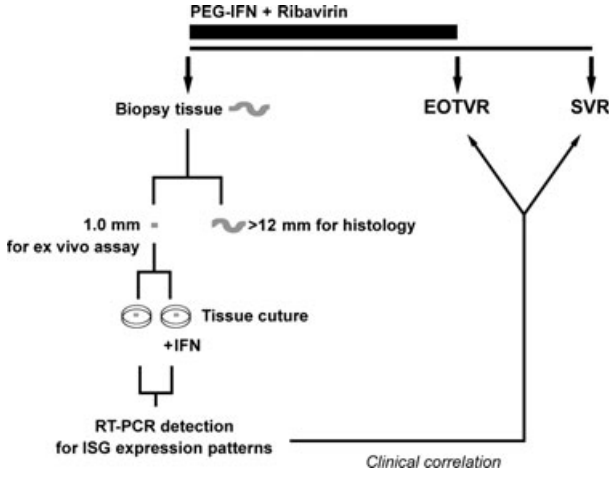

Fig. 1 Experimental design and strategy. PEG-INF, peginterferon; EOTVR, end of therapy virological response; SVR, sustained virological response; IFN, interferon.

bovine serum. When the cells reached 80% confluence, two equally sized liver slices derived from the same patient were cocultured with the Vero cells. Interferon (5000 IU/mL) was added into the culture medium of one of the liver slices. The liver slices were harvested at 24 h, after coculture. The tissues were subjected to RT-PCR assay for the assessment of ISG expression.

RT-PCR for the detection of ISG expression

RNA extraction was performed using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA). RT was performed using random primers. PCR was performed for 30 cycles (95°C for 1 min, 55°C for 1 min and 72°C for 1 min) in a DNA thermal cycler (Perkin-Elmer Cetus, Emeryville, CA, USA) [22, 23].

To quantitatively assess the mRNA levels of ISGs, real-time RT-PCR assay was performed in the LightCycler (Roche Diagnostics Corporation, Indianapolis, IN, USA). The procedure was described previously [19]. The abundance of ISG mRNA levels was normalized to the β-actin mRNA levels in the same biopsy tissues. The relative unit (RU) of ISG mRNAs was calculated by assigning the level as 1 RU in a patient positive for all ISG mRNA expression prior to interferon treatment. The RU values of ISG mRNAs in all other patients were calculated as the folds of expression levels relative to those in the assigned patient.

Immunohistochemistry analysis

When a larger piece of liver biopsy tissue was obtained, immunohistochemistry was also performed following liver slice culture in the presence or absence of interferon treatment. The method of immunohistochemistry was described previously. Anti-OAS antibody (OAS1 (V-18): sc-49849; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used to detect OAS1 expression in the liver tissues.

Statistical analysis

Results were given as mean ± SD or median (range) for nonparametric data. Analysis was conducted using two-sample *t*-test, Mann–Whitney test, Chi-squared test with Yates' correction and Fisher's exact test where appropriate. All statistical testing was two-tailed at the 5% level. Univariate analysis was performed using logistic regression model. Stepwise multivariate logistic regression model was conducted to determine independent factors associated with the end of therapy virological response or SVR. The overall model fit was examined by likelihood function. The statistical analysis was conducted using the Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA) version 13.0.

RESULTS

Experimental design

To understand the role of hepatic ISG expression in interferon-based antiviral therapy against chronic hepatitis C, 104 consecutive patients were included. The basic clinical data were listed in Table 1. All patients received liver biopsy prior to a standard course of peginterferon plus ribavirin therapy. A small piece of liver biopsy sample was divided into two portions for *ex vivo* culture (Fig. 1). One of them was treated with interferon for 24 h. Both samples were then harvested for RT-PCR detection of the ISG expression. The ISG expression patterns as well as all clinicopathological data were correlated with the outcomes of subsequent antiviral treatment.

Detection of ISGs by RT-PCR

The primers used for detection were listed in Table 2. Totally, 8 ISGs were detected, including PRKR, interferon regulatory factor 9 (IRF9), interferon regulatory factor 1 (IRF1), interferon-inducible guanylate binding protein 1 (GBP1), interferon alpha-inducible protein 6 (IFI6), interferon alpha-inducible protein 27 (IFI27), MxA and OAS1. After RT-PCR, three patterns were identified: noninducible pattern (NI), inducible pattern (I) and previously induced pattern (PI). Chronic hepatitis C patients with NI pattern had liver tissues that did not express the target ISG before and after interferon treatment. Patients with I pattern had liver tissues that did not express the target ISG before interferon treatment but expressed a detectable level of ISG after interferon treatment. Patients with PI pattern had liver tissues that expressed a detectable level of target ISG before and after interferon treatment, indicating that the ISG had

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Table 2 Primers used for interferon-inducible gene expression assays

Target gene	GenBank accession No.	Primers	Size of RT-PCR product (nt.)
Interferon-inducible double-stranded RNA-dependent protein kinase R (PRKR)	AK313818	PKR-1 5′-gtgattatgatcctgagaac-3′ (nt. 1111–1130; sense) PKR-2 5′-ctttccatagtcttgcgaag-3′ (nt. 1481–1462; antisense)	371
Interferon regulatory factor 9 (IRF9)	NM_006084	IRF9-1 5′-aactcaggatggcatcaggc-3′ (120–139; sense) IRF9-2 5′-agacagctggacctcctgtg-3′ (365–346; antisense)	246
Interferon regulatory factor 1 (IRF1)	NM_002198	IRF1-1 5′-aagaggagatgatcttccag-3′ (320–339; sense) IRF1-2 5′-tcacctcctcgatatctggc-3′ (517–498; antisense)	198
Interferon-inducible guanylate binding protein 1 (GBP1)	NM_002053	GBP1-1 5′-ctgatggcgaatccagaagc-3′ (281–300; sense) GBP1-2 5′-ctaggatgtggcctggcttc-3′ (500–481; antisense)	220
Interferon alpha-inducible protein 6 (IFI6)	BC015603	IFI6-1 5′-tgctacctgctgctcttcac-3′ (107–126; sense) IFI6-2 5′-aggcagccaccgagttggcc-3′ (296–277; antisense)	190
Interferon alpha-inducible protein 27 (IFI27)	BC015492	IFI27-1 5′-catcagcagtgaccagtgtg-3′ (211–230; sense) IFI27-2 5′-agtgactgcagagtagccac-3′ (460–441; antisense)	250
Myxovirus (influenza virus) resistance 1, interferon-inducible protein 78 (MxA)	BC032602	MxA-1 5′-cccttcccagaggcagcggg-3′ (572–591; sense) MxA-2 5′-ctgattgcccacagccactc-3′ (861–842; antisense)	290
2′,5′-oligoadenylate synthetase 1, 40/46kDa (OAS1)	BT006785	OAS-1 5′-ggtggtaaagggtggctcctc-3′ (171–191; sense) OAS-2 5′-tctgcaggtcggtgcactcc-3′ (544–525; antisense)	374

already been induced during chronic HCV infection. The representative RT-PCR data were shown in Fig. 2. Notably, a doublet RT-PCR product was found for IFI27 suggesting coexistence of two variants.

Association between ISGs and the therapeutic responses

To understand whether ISG expression was correlated with treatment outcomes, univariate logistic regression analysis was performed for all clinicopathological factors and ISG expression patterns (Table 3; See Table S1 and S2 for details). It was found that AST/ALT ratio ($P = 0.042$), leucocyte count ($P = 0.014$), HCV-RNA level ($P = 0.014$) and MxA expression patterns (PI pattern, $P = 0.016$ and I + PI pattern combined, $P = 0.006$) were associated with the end of therapeutic virological response. Multivariate logistic regression analysis was thus performed to identify independent predictors. It was found that MxA expression (I + PI pattern; OR, 16.904; 95% CI, 2.069–138.113; $P = 0.008$) and leucocyte count (OR, 0.652; 95% CI, 0.433–0.981; $P = 0.040$) were independently associated with the end of therapeutic virological response.

On the other hand, univariate analysis showed that AST/ALT ratio ($P = 0.012$), HCV-RNA level ($P = 0.015$), genotype 1 ($P < 0.001$) and OAS1 expression patterns (PI pattern, $P = 0.007$ and I + PI pattern combined, $P = 0.001$) were associated with the SVR. Multivariate logistic regression analysis was thus performed to identify independent predictors. It was found that OAS1 expression (I + PI pattern; OR, 29.499; 95% CI, 3.193–272.534; $P = 0.003$), leucocyte count (OR, 0.640; 95% CI, 0.424–0.967; $P = 0.034$), genotype 1 (OR, 0.086; 95% CI, 0.018–0.409; $P = 0.002$), HCV-RNA level (OR, 0.396; 95% CI, 0.203–0.772; $P = 0.007$) and AST/ALT ratio (OR, 0.025; 95% CI, 0.002–0.318; $P = 0.004$) were independently associated with the SVR.

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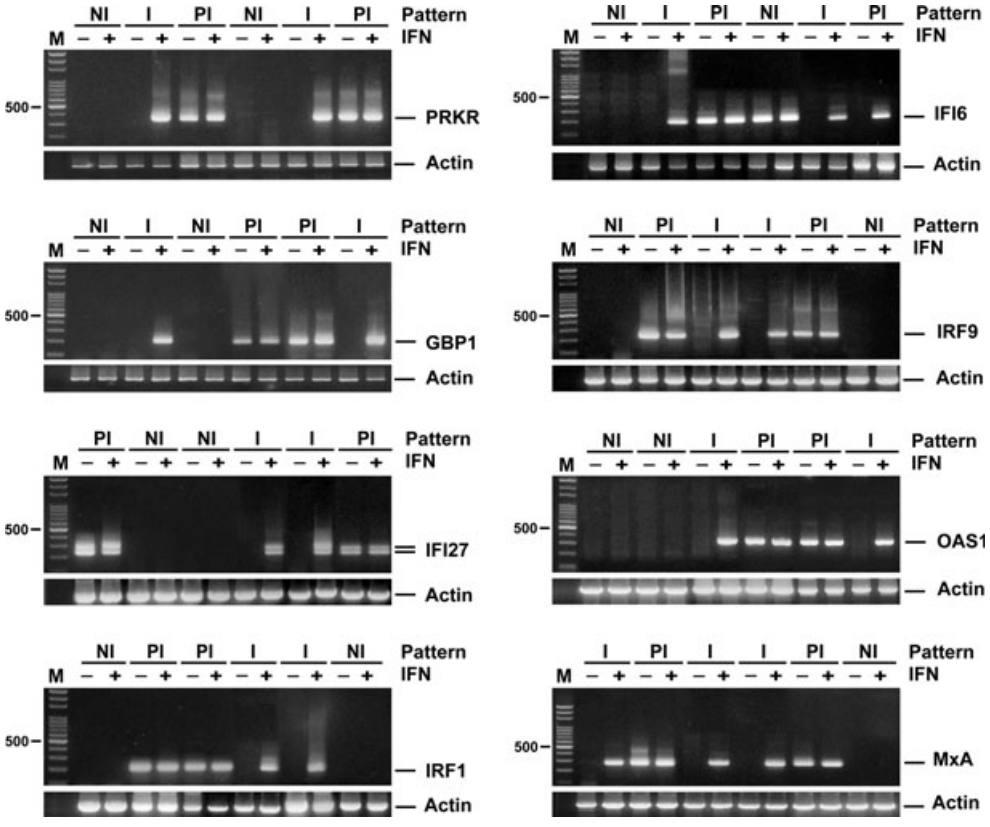

Fig. 2 Expression patterns of ISGs in the presence (+) or absence (−) of interferon (IFN) in the *ex vivo* culture medium. NI, noninducible; I, inducible; PI, previously induced.

Interferon-induced expression of OAS1 in liver tissues

Because OAS1 expression was an important independent factor for the SVR, we examined the location of OAS1 expression in the liver tissues. There remained a remote possibility that the OAS1 expression observed in liver slice culture was in fact originated from infiltrating lymphocytes. Immunofluorescence analysis was performed in 9 pairs of cultured liver slice tissues. It was showed that the OAS1 expression indeed expressed in the liver tissues, consistent with the patterns obtained from RT-PCR (Fig. 3).

Quantitative assessment for ISG expression levels prior to interferon treatment by real-time RT-PCR

To understand whether the expression levels in the biopsy samples prior to interferon treatment were associated with the treatment outcomes, quantitative assessment was performed for all ISG expression in the absence of interferon. The relative abundance of ISG mRNAs was represented by the levels of relative unit (RU) as described in the Materials and Methods. It was discovered that a high pretreatment level of IRF9, IFI6 and MxA was associated with failure to achieve the sustained viral response ($P = 0.032$, 0.040 and 0.021, respectively; logistic regression). For other ISGs, no significant association was found between the expression level and treatment outcomes. Multivariate analysis showed that the three pretreatment ISGs were not independently associated with SVR.

DISCUSSION

It is generally believed that the viral suppression effect exerted by interferon is mainly mediated by ISG expression [24–26]. Of the functionally characterized ISGs, MxA, OSA1 and PRKR have a clear suppression role for virus replication in several infectious diseases [27–29]. However, the link between the therapeutic interferon-induced ISG expression and the subsequent virological responses has not been clearly established, particularly in chronic HCV infection. Only limited data are available to show that interferon-mediated ISG expression indeed occurs in human liver cells but not in the hepatic lymphocytes, and basically, no data are available to connect the on-treatment ISG responses in human liver (i.e. paired biopsies before and after interferon treatment) and the final therapeutic outcomes (i.e. SVR). As a result, one does not know which ISGs are the key factors required for effective interferon-based antiviral therapy.

Some studies were conducted by evaluating ISGs production in peripheral blood mononuclear cells, but it is doubtful

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Table 3 Univariate analysis for the association of sustained virological response with pretherapeutic clinical factors and *ex vivo* OAS1 expression profiles in chronic hepatitis C patients receiving interferon therapy

	Sustained virological response		OR (95% CI)	*P*
Parameters	Yes	No		
Number of patients	78	26		
Sex, Male	43	13	1.229 (0.505–2.988)	0.650
Age, years	50.7 ± 10.1	55.5 ± 11.9	0.956 (0.913–1.001)	0.055
Body mass index	25.5 ± 4.0	25.4 ± 2.8	1.001 (0.998–1.003)	0.935
Biochemistry				
AST, U/L	103.3 ± 64.1	95.5 ± 50.6	1.002 (0.995–1.010)	0.569
ALT, U/L	170.2 ± 118.8	123.0 ± 57.0	1.006 (1.000–1.012)	0.059
AST/ALT	0.66 ± 0.24	0.87 ± 0.48	0.167 (0.041–0.679)	**0.012**
Bilirubin, mg/dL	0.9 ± 0.4	0.8 ± 0.4	1.720 (0.443–6.671)	0.433
Alpha-fetoprotein, ng/mL	13.1 ± 16.7	13.4 ± 16.2	0.999 (0.973–1.026)	0.930
Albumin, g/dL	4.5 ± 0.4	4.3 ± 0.6	1.893 (0.767–4.670)	0.166
White blood cells				
Total, $\times 10^3$	5.50 ± 1.23	6.00 ± 2.01	0.798 (0.593–1.075)	0.138
Neutrophil (%)	51.7 ± 9.9	52.6 ± 6.8	0.990 (0.943–1.039)	0.668
HCV-RNA, MIU/mL	0.54 ± 0.78	1.19 ± 1.48	0.571 (0.363–0.898)	**0.015**
HCV genotype 1	32	22	0.126 (0.040–0.402)	**<0.001**
Histology activity index	7.5 (3–14)	8 (4–14)	0.902 (0.744–1.093)	0.293
Fatty liver, Yes	32	7	1.888 (0.711–5.016)	0.202
OAS1, NI/I/PI*				
NI	39	24		
I	12	1	7.385 (0.902–60.450)	0.062
PI	27	1	16.615 (0.2118–130.323)	**0.007**
I + PI	39	2	12.000 (2.653–54.279)	**0.001**

*NI, noninducible; I, inducible; PI, previously induced. Bold values, $P < 0.05$.

whether these results could be extrapolated to interferon effects in the liver [16]. Another study assessed ISGs production in pretreatment liver biopsies [17]. However, a significant variation of pretreatment ISG level was observed and the response of ISGs production upon interferon treatment could not be assessed, which was the key parameter to be evaluated, linking the interferon-induced ISG response to treatment outcome. To our knowledge, paired liver biopsies (before and after interferon treatment) were performed for evaluation in only one study in a limited number of patients (n = 16) [18]. In that study, the ISG responses were only correlated with rapid responders and nonrapid responders (evaluated in week 4).

By performing gene profiling analysis in pretreatment liver biopsy tissues, it was found that increase of some ISGs greater than mean levels (before treatment) was associated with nonresponsiveness to pegylated interferon plus ribavirin therapy [30,31]. These studies led to the hypothesis that if high levels of ISGs were induced by HCV infection but failed to eliminate the viruses, nonresponsiveness could be expected in the subsequent interferon-based therapy. In the present study, ISGs expression was indeed induced before interferon therapy in a large proportion of patients. Statistically, when noninducers (NI) group was used as a reference, the presence of pretherapeutic ISG induction (MxA and OAS1; the PI group) was associated with favourable treatment outcomes. The association was even stronger when the group of patients with interferon-induced ISG responses (I group) was included. Importantly, the MxA and OAS1 expression were identified as independent outcome predictors in a multivariate model including other well-established predictors (HCV-RNA and genotype), suggesting a pivotal role for their antiviral activities. In this study, paired expression data (with or without interferon treatment) were available for analysis and the noninducers were used as references, whereas in the gene profiling studies, only single (pretreatment) biopsy data were applied. In our patients, when only the baseline ISG expression levels were assessed by real-time RT-PCR for outcome correlation, high expression levels of IRF9, IFI6 and MxA were associated with failure of the SVR. This result was consistent with previous studies using only pretreatment liver biopsies for correlation [30–32]. However, in this study, pretreatment expression levels of OAS1 were not significantly associated with treatment outcomes. The present data indicated that results derived from paired liver tissues could provide new information and thus new insight into this issue.

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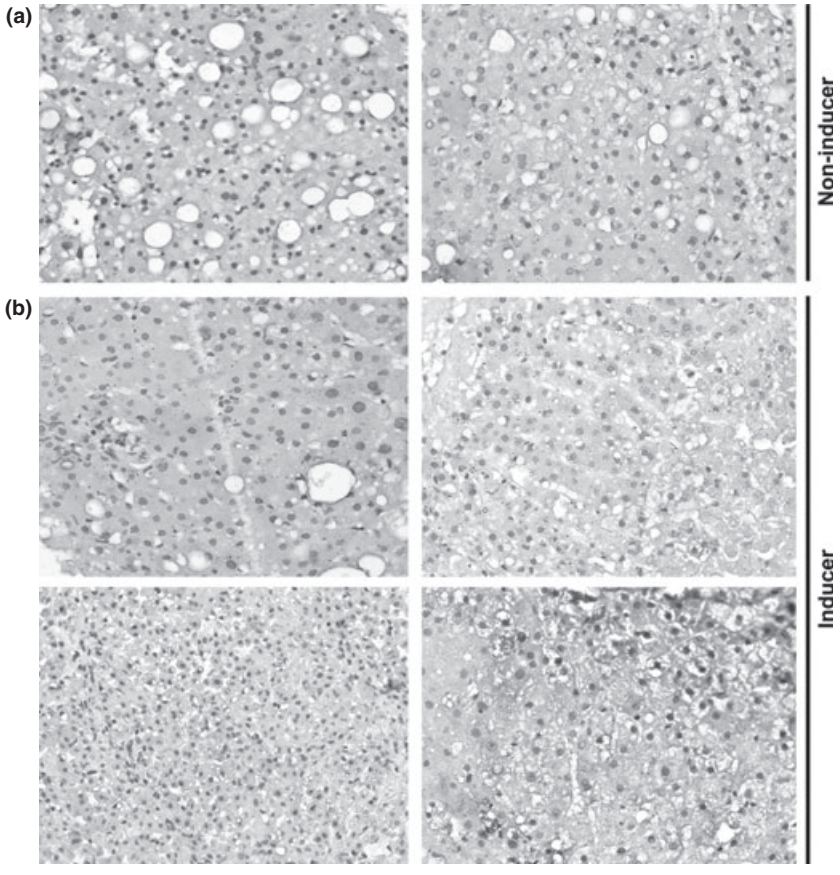

Fig. 3 Immunohistochemistry analysis of OAS1 expression in hepatocytes. (a) OAS1 expression in the liver tissues obtained from an OAS1 noninducer in the absence (left panel) or presence (right panel) of interferon. (b) OAS1 expression in the liver tissues obtained from an OAS1 inducer in the absence (left upper panel) or presence (right upper and lower panels) of interferon. All panels were shown at 400 × magnification except for left lower panel (200 × magnification).

In this study, it was found that MxA expression was associated with the end of therapeutic virological response but not SVR, whereas the OAS1 expression was associated with SVR but not the end of therapeutic virological response. The reason for this differential effect is not clear. We speculate that when the viral load is high, MxA has a stronger antiviral effect compared with OAS1, possibly because of some unrecognized anti-ISGs mechanisms exerted by HCV viral proteins, which preferentially suppress the antiviral function of OSA1. On the other hand, after HCV is largely cleared, OAS1 become a better suppressor for HCV replication in the absence of the anti-ISGs machineries. Recently, it was found that IL28B (interferon lambda) genotype was strongly associated with treatment outcomes. It can be speculated that interferon lambda expression as well as the associated ISG induction was pivotal to the outcome of HCV infection. It was unclear at this time whether the IL28B genotype and ISG expression were independent predictors for treatment outcomes. The clinical trials to use interferon lambda to treat chronic hepatitis C patients are now in progress.

The expression pattern of PRKR is not correlated with therapeutic outcomes in this study. A possible explanation for this observation is that the activities changes rather than the alterations of expressional levels of PRKR are more important in predicting treatment outcomes in chronic hepatitis C patients receiving interferon-based treatment. Alternatively, it is also possible that PRKR does not play an important role in antiviral therapy of chronic hepatitis C.

In conclusion, using an *ex vivo* liver slice culture assay, expression of ISGs in liver tissues before and after interferon treatments can be demonstrated. Hepatic expression of MxA and OAS1 independently predicts the end of therapy virological response and SVR, respectively, in the subsequent antiviral therapy.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1: Univariate analysis for association of end-of-therapy response with pre-therapeutic clinical factors and *ex vivo* ISG gene expression profiles in chronic hepatitis C patients receiving interferon therapy.

Table S2: Univariate analysis for association of sustained virological response with pre-therapeutic clinical factors and *ex vivo* ISG gene expression profiles in chronic hepatitis C patients receiving interferon therapy.

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