表4 原因血液と患者の背景

症例	報告年	患者成績				献血者成績			
		年齢(性)	原疾患	ALT(IU/L) MAX.	genotype	個別NAT	genotype	HCV抗体	年齢(性)
1	1996	46(男)	S状結腸癌	581	2a	+	2a	−	23(女)
2	1997	70(女)	急性リンパ性白血病	254	1b	+	1b	−	26(男)
3	1998	63(男)	骨髄異型成症候群	123	1b	+	1b	−	19(女)
4	1998	60(男)	出血性胃潰瘍	81	2a	+	2a	−	34(男)
5	1998	56(男)	胃癌	221	2a	+	2a	−	34(男)
6	1998	32(男)	広範囲熱傷	245	N.T	+	1b	−	23(女)
7	1998	65(男)	十二指腸出血	211	2a	+	2a	−	48(男)
8	1998	78(女)	急性骨髄性白血病	107	1b	+	1b	−	29(女)
9	1998	63(男)	右大腿骨頸部骨折	308	1b	+	1b	−	49(女)
10	1999	48(男)	多発性骨髄腫	11	2b	+	2b	−	32(女)
11	1999	75(男)	肝細胞癌	298	2b	+	2b	−	38(男)
12	1999	38(女)	再生不良性貧血	46	1b	+	1b	−	20(女)
13	1999	48(男)	胃潰瘍	903	1b	+	1b	−	27(男)
14	1999	69(男)	多発性骨髄腫	69	N.T	+	N.T	−	37(男)
15	2005	81(女)	骨髄異型成症候群	407	1b	+	1b	−	44(女)
16	2006	35(女)	常位胎盤早期剥離	12	2a	+	2a	−	21(女)
17	2007	54(女)	再生不良性貧血	26	2a	+	2a	−	22(男)

症例4，5の献血者は同一
上記以外に初流血除去・保存前白血球除去も導入済み

医療機関から報告されてくるが，特定される例は極めて少なく，輸血とは別経路のHCV感染を疫学的に調査することが必要ではないかと思われる．

6. イギリスでの現状(SHOTより)

輸血による肝炎は2004年にHEVの1例，2005年にHBVの1例が同定されているが，それ以降は，細菌感染が主な原因となっている．

(from http://www.shotuk.org/shot-reports/reports-and-summaries-2001-2002/)

7. 血液製剤によるHCV感染

血液製剤でHCV感染が問題となったのは，非加熱製剤である．最近では，過去にフィブリノゲン製剤，血液凝固因子製剤を投与された患者で問題となった．免疫グロブリン製剤でもバクスター社のGammagardに関連したHCV感染が報告され，HCVを不活化するS/D処理が製造工程に導入された経緯もある[17]．その後，血漿分画製剤についてはウイルスの除去・不活化工程を2種以上導入することが義務付けられ，少なくとも，HBV・HCV・HIVの製造工程中のウイルスクリアランス指数が10^9以上を担保しなくてはいけないことになっている．また，国内原料血漿については，NAT導入によりウイルス混入量が極限まで少なくなっている．更に，2010年度中にはHIV，HBV，HCVを検出するNATの感度がHBV-DNA(2,000 IU/mL)，HCV-RNA(2,000 IU/mL)，HIV-RNA(4,000 IU/mL)と規定される予定であるが(血液製剤のウイルスに対する安全性確保を目的としたNATに必要とされる検出限界値について．平成22年10月6日付厚生労働省医薬食品局血液対策課よ

り)，日赤のNATはこれを上回る感度で実施しているので問題はない．つまり，現在，市場に流通している血液製剤によってHCV感染は起こらない．

8. ま と め

献血血液に対するNATの導入と改善によって輸血によるC型肝炎は激減し，許容できるリスクとなった．しかし，ゼロリスクではなく現行のNATシステムでもすり抜けるWPの血液は否定できない．医療機関側では，輸血とは別の感染経路を調査すると供に，HCV感染の早期検出のためにも抗体検査からc抗原へ変更することが肝要である．

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Open Access Full Text Article ORIGINAL RESEARCH

Type I interferon receptor in peripheral blood mononuclear cells may predict response to intra-arterial 5-fluorouracil + interferon therapy for advanced hepatocellular carcinoma

Yasuyuki Tomiyama[1]
Naoko Yoshioka[1]
Yoshiaki Yanai[2,3]
Tomoya Kawase[1]
Sohji Nishina[1]
Yuichi Hara[1]
Koji Yoshida[1]
Keiko Korenaga[1]
Masaaki Korenaga[1]
Keisuke Hino[1]

[1]Department of Hepatology and Pancreatology, Kawasaki Medical University, Kurashiki, Japan; [2]Institute of Fujisaki, Hayashibara Biochemical Lab Inc, Okayama, Japan; [3]Pharmaceutical Marketing Division, Otsuka Pharmaceutical Co Ltd, Tokyo, Japan

Background: Type 1 interferon alpha receptor 2 (IFNAR2) in the liver has been reported to be a predictive factor for the response to intra-arterial 5-fluorouracil (5-FU) + systemic interferon (IFN)-alpha combination therapy in patients with advanced hepatocellular carcinoma. We tested whether IFNAR2 expression in peripheral blood mononuclear cells could predict the response to 5-FU + IFN.

Methods: Predictive factors for survival and response to therapy were determined in 30 patients with advanced hepatocellular carcinoma who underwent treatment with 5-FU + IFN. IFNAR2 expression in peripheral blood mononuclear cells was measured in 11 of the 30 patients.

Results: With a mean number of 4.2 courses of combination therapy, one patient (3%) showed a complete response, eight (27%) showed partial responses, 13 (43%) had stable disease, and eight (27%) showed progressive disease. The median survival time of responders (complete response/partial response) was 12.7 months and that of nonresponders (stable disease/progressive disease) was 7.5 months. The one-year and two-year cumulative survival rates of responders and nonresponders were 87/69% and 40/11%, respectively ($P = 0.019$). Multivariate analysis identified response to therapy ($P = 0.037$) as the sole independent determinant of survival. The expression level of IFNAR2 in peripheral blood mononuclear cells was significantly ($P = 0.012$) higher in responders (6.5 ± 2.4) than in nonresponders (2.4 ± 0.6), even though no clinical factors were identified as being associated with the response to the combination therapy.

Conclusion: IFNAR2 expression in peripheral blood mononuclear cells may predict the response to 5-FU + IFN therapy in patients with advanced hepatocellular carcinoma, although these data are preliminary.

Keywords: interferon, 5-fluorouracil, hepatocellular carcinoma, receptor

Introduction

Hepatocellular carcinoma is the third leading cause of cancer-related death globally, behind lung and stomach cancers.[1] Its incidence has been increasing in Japan in the last 30 years[2] and also in the US more recently.[3] Intensive management of patients at high risk for hepatocellular carcinoma and advances in diagnostic techniques have facilitated the detection of hepatocellular carcinoma in the early stage.[4–7] Simultaneously, several therapeutic modalities, including hepatic resection, liver transplantation, radiofrequency ablation, percutaneous ethanol injection, and transcatheter arterial chemoembolization have substantially improved the prognosis of patients with hepatocellular carcinoma.[8–11] Nevertheless, we still sometimes see patients with advanced hepatocellular carcinoma

Correspondence: Keisuke Hino
Department of Hepatology and Pancreatology, Kawasaki Medical University, 577 Matsushima, Kurashiki 701-0192, Japan
Tel +818 6462 1111
Fax +818 6464 1196
Email khino@med.kawasaki-m.ac.jp

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at their first visit or after repeated treatment due to the frequent recurrence of the disease.

The prognosis of patients with advanced hepatocellular carcinoma, especially if complicated by portal venous invasion, is extremely poor.[12,13] Recently, sorafenib, an oral multikinase inhibitor of the vascular endothelial growth factor receptor, the platelet-derived growth factor receptor, and Raf, has been shown to prolong median survival time and the time to progression by nearly three months in patients with advanced hepatocellular carcinoma as compared with those given placebo.[14] However, no complete responses and only a few partial responses (2%) were found in the same study. Although sorafenib can be used for the treatment of patients with advanced hepatocellular carcinoma, its clinical effectiveness is still controversial in Japan. According to the consensus-based clinical manual proposed by the Japan Society of Hepatology,[15] arterial infusion chemotherapy using an implantable drug delivery system is recommended as one of the treatments for advanced hepatocellular carcinoma with portal venous invasion, based on the favorable results of combination therapy with intra-arterial 5-fluorouracil (5-FU) + systemic interferon (IFN)[16–19] or another combination of low-dose cisplatin + 5-FU.[20,21] To improve the effects of these combination therapies and to increase the response rates, it is important to find a practical and useful predictor of the response to therapy. Hepatic expression of type I interferon alpha receptor 2 (IFNAR2) has been shown to correlate with the response to 5-FU + IFN in patients with advanced hepatocellular carcinoma and portal venous invasion.[17] However, liver biopsy is sometimes difficult to perform before combination therapy in patients with advanced hepatocellular carcinoma because of a bleeding tendency with a low count platelet and/or decreased activity of prothrombin. In this pilot study, we tested whether IFNAR2 expression in peripheral blood mononuclear cells could predict the response to 5-FU + IFN in patients with advanced hepatocellular carcinoma.

Materials and methods

Patients

A single-arm, open-label study of intra-arterial combination therapy was conducted in patients with advanced hepatocellular carcinoma. Eligibility criteria were as follows: hepatocellular carcinoma with tumor thrombi invading at least one of the major branches of the portal vein (Vp3 or Vp4, according to the criteria of the Liver Cancer Study Group of Japan [LCSGJ]) or multiple intrahepatic metastases in more than three segments, irrespective of the degree of portal venous invasion (Vp1, Vp2, Vp3, or Vp4); tumor staging III or IVA based on the TNM (tumor node metastasis) staging system of the LCSGJ; absence of extrahepatic metastases; Child–Pugh A or B liver function; an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1;[22] leukocyte count >2000/μL; platelet count >50,000/μL; unresectable disease or not suitable for local ablation therapy; and unlikelihood of effectiveness with transcatheter arterial chemoembolization. All patients provided written informed consent for this study, which was approved by the institutional review board of Kawasaki Medical University.

Implantation of intra-arterial catheter

An indwelling intra-arterial catheter (Piolax W spiral catheter, Piolax Medical Devices Inc, Kanagawa, Japan) was inserted through the femoral artery by the Seldinger method, and its tip was put in the proper hepatic artery or common hepatic artery, embolizing the right gastric and gastroduodenal arteries to avoid efflux of chemotherapeutic agents into the stomach and duodenum. The other end of the catheter was connected to the injection port (Vital-Port, Cook Japan, Tokyo, Japan) subcutaneously implanted in the lower abdomen.

Evaluation of response to therapy

The response to therapy was assessed with contrast-enhanced computed tomography after each therapeutic cycle. The response was defined according to the Response Evaluation Criteria in Solid Tumors (RECIST)[23] as: complete response (complete disappearance of all target lesions); partial response (at least a 30% decrease in the sum of the longest diameter of the target lesions), taking as reference the baseline sum of the longest diameter; progressive disease (at least a 20% increase in the sum of the longest diameter of target lesions or the appearance of one or more new lesions); or stable disease (neither partial response nor progressive disease criteria). The best response to therapy was defined as the response to therapy when a different response, such as partial response or stable disease, was found in the same patient during multiple treatment cycles. Adverse reactions were assessed using the National Cancer Institute Common Toxicity Criteria (NCI-CTC, version 3.0, [http://ctep.cancer.gov/reporting/ctc.html]).

Treatment protocol

One cycle of treatment consisted of four weeks in which 5×10^6 U (5 MU) of IFN-alpha (OIF; Otsuka Pharmaceutical, Tokyo, Japan) was administered intramuscularly

on days 1, 3, and 5 of each week, resulting in a total dose of 60 MU per cycle. 5-FU (Kyowa Hakko, Tokyo Japan) 500 mg/day was administered into the hepatic artery over five hours using a portable infusion pump on days 1–5 of the first and second weeks (5 g per cycle). The combination therapy was discontinued in patients who did not meet the eligibility criteria and also in those with progressive disease or NCI-CTC Grade 3 adverse reactions, otherwise the treatment was repeated after a 2–4-week rest period without treatment.

Measurement of IFNAR2 expression in peripheral blood mononuclear cells

Peripheral blood mononuclear cells were separated from 10 mL of heparinized blood by density gradient centrifugation using Ficoll-Hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden), washed three times with RPMI 1640 culture medium, and stored at −80°C until use. RNA was extracted from the homogenized peripheral blood mononuclear cells using a High Pure RNA kit (Roche Diagnostics Ltd, Germany), and its integrity was confirmed by spectrophotometry. The IFNAR2 mRNA expression level was quantified using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) and real-time reverse transcription polymerase chain reaction (RT-PCR), as described previously.[24] Briefly, a RT-PCR assay was performed on a 25 μL reaction mixture containing 20 ng of sample cDNA, 100 nM sense primer, 100 nM antisense primer, and 12.5 μL of SYBR Green PCR Master Mix (Applied Biosystems). The following specific primers were designed to amplify their respective genes; IFNAR2, sense; 5′-GAAGGTGGTTAAGAACTGTGC-3′, antisense; 5′-CCCGCTGAATCCTTCTAGGACGG-3′; β2-microglobulin, sense; 5′-ACCCCCACTGAAAAAGATGA-3′, antisense; 5′-ATCTTCAAACCTCCATGATG-3′. The PCR was carried out for 45 cycles at 95°C for 15 seconds and 60°C for one minute. A standard curve for each mRNA expression was generated using five-fold dilutions of a control RNA sample (25×, 5×, 1×, 0.2×, and 0.04×). The mRNA expression levels of the target genes (IFNAR2) were presented as a ratio to that of β2-microglobulin, and the relative expression levels were calculated.

Statistical analysis

Quantitative values were expressed as the mean ± standard deviation. Cumulative survival was calculated using the Kaplan–Meier method, and the differences between the groups were analyzed using the log-rank test. Univariate and multivariate analyses of predictors of survival were assessed by the Cox proportional hazards model. Univariate and multivariate analyses of predictors for the response to therapy were assessed by the logistic regression test. Differences between the two groups were examined for statistical significance using the Mann–Whitney U test. A P value < 0.05 was considered to be statistically significant. All analyses described above were performed using SPSS software (version 11, SPSS Inc, Chicago, IL).

Results

Patient profile

Forty-five patients with advanced hepatocellular carcinoma fulfilled the eligibility criteria for 5-FU + IFN therapy. Among them, 30 patients (24 men and six women) with an average age of 64.7 ± 1.8 (range 48–84) years provided written informed consent to receive the combination therapy. Patient characteristics at baseline are shown in Table 1. Eight patients were positive for both hepatitis B (HBV) surface antigen and HBV DNA, and 18 for both anti-hepatitis C virus (HCV) and HCV RNA. The remaining four patients were negative for both hepatitis B surface antigen and anti-HCV. Liver disease stage was Child–Pugh A and tumor stage was IV in 23 patients (76.7%). The integrated staging scores for the Japan Integrated Staging[25] and Cancer of the Liver Italian Program (CLIP)[13] were ≥3 in 23 (76.7%) and 17 patients (56.7%), respectively. Twelve patients (40%) had portal venous invasion at a major branch (Vp3) or in the main trunk (Vp4).

Table 1 Patient characteristics

Number of patients	30
Age, years, mean ± SD (range)	64.7 ± 1.77 (48–84)
Gender, male/female	24/6
Etiology (HBV/HCV/NBNC)	8/18/4
Total bilirubin (mean ± SD, mg/dL)	1.1 ± 0.1
Albumin (mean ± SD, g/dL)	3.5 ± 0.08
Prothrombin time (mean ± SD, %)	77.2 ± 2.2
Platelet count (mean ± SD, $\times 10^4/\mu L$)	14.7 ± 1.4
AFP (mean ± SD, ng/mL)	33,715 ± 13,255
AFP-L3 (mean ± SD, %)	23.1 ± 4.6
DCP (mean ± SD, mAU/mL)	37,905 ± 17,417
Child–Pugh status (A/B/C)	23/7/0
TNM staging by LCSGJ (III/IVA)	7/23
JIS score (1, 2/3, 4, 5)	7/23
CLIP score (1, 2/3, 4, 5)	13/17
Portal vein invasion (Vp1 or Vp2/Vp3 or Vp4)	18/12

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; NBNC, non-HBV non-HCV; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; TNM, tumor node metastasis; LCSGJ, Liver Cancer Study Group of Japan; JIS, Japan integrated staging; CLIP, Cancer of the Liver Italian Program; SD, standard deviation.

Response to combination therapy and survival

Thirty patients with advanced hepatocellular carcinoma completed 5-FU + IFN therapy, with a mean treatment cycle number of 4.2 (range 2–12). The median survival time was 7.5 months, and the one-year and two-year cumulative survival rates were 53% and 33%, respectively. Of these 30 patients, one (3%) had a complete response, eight (27%) had a partial response, 13 (43%) had stable disease, and 8 (27%) had progressive disease, ie, nine (30%) had objective responses (complete response or partial response). The median survival time of responders (complete response/partial response) was 12.7 months and that of nonresponders (stable disease/progressive disease) was 7.5 months. The one-year and two-year cumulative survival rates for responders and nonresponders were 87%/69% and 40%/11%, respectively. Thus, there was a significant difference in the overall survival rate between responders and nonresponders ($P = 0.019$, Figure 1).

Factors associated with survival

We investigated the predictors of survival in patients who underwent 5-FU + IFN therapy. Univariate analysis identified total bilirubin concentration ($P = 0.005$), CLIP score ($P = 0.019$), and response to therapy ($P = 0.033$) as factors associated with survival (Table 2). Among these factors, multivariate analysis identified the response to therapy ($P = 0.037$) as a significant and independent determinant of survival (Table 3).

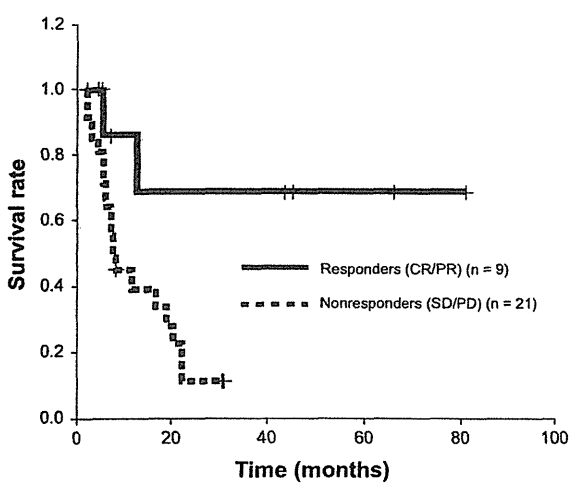

Figure 1 Comparison of overall survival rates of responders (complete response or partial response) and nonresponders (stable disease or progressive disease) to 5-FU + IFN therapy. The survival rate was significantly higher in responders than in nonresponders (log-rank test, $P = 0.019$).
Abbreviations: 5-FU, 5-fluorouracil; IFN, interferon.

Table 2 Univariate analysis of predictors for survival

Variable	Hazards ratio	95% CI	P value
Age	0.956	0.907–1.007	0.091
Male	2.675	0.694–10.311	0.153
HBsAg positive	0.460	0.169–1.249	0.128
Anti-HCV positive	1.503	0.604–3.735	0.381
Total bilirubin (mg/dL)	3.222	1.420–7.313	0.005
Albumin (g/dL)	0.413	0.143–1.193	0.102
Prothrombin time (%)	0.964	0.917–1.014	0.160
Platelet count ($\times 10^4/\mu L$)	0.976	0.918–1.036	0.976
AFP (<100 ng/mL)	1.372	0.551–3.416	0.497
AFP-L3 (<20%)	1.509	0.610–3.731	0.373
DCP (<100 mAU/mL)	0.445	0.101–1.954	0.283
Child–Pugh status A	2.549	0.950–6.843	0.063
Tumor stage III	2.995	0.858–10.460	0.086
JIS score (<3)	2.995	0.858–10.460	0.086
CLIP score (<3)	3.421	1.222–9.576	0.019
Portal vein invasion (<Vp3)	2.288	0.871–6.010	0.093
Response to therapy (CR or PR)	4.960	1.136–21.668	0.033

Abbreviations: CI, confidence interval; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; NBNC, non-HBV non-HCV; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; TNM, tumor node metastasis; LCSGJ, Liver Cancer Study Group of Japan; JIS, Japan Integrated Staging; CLIP, Cancer of the Liver Italian Program; CR, complete response; PR, partial response.

Factors associated with response to combination therapy

We examined factors associated with the response to 5-FU + IFN therapy, because response to therapy was found to be the only independent factor associated with survival in patients who underwent treatment with this combination. However, univariate and multivariate analyses did not identify any significant factors associated with response to the combination therapy (Table 4).

IFNAR2 in peripheral blood mononuclear cells and response to 5-FU + IFN

To explore factors associated with the response to the combination treatment, we next measured IFNAR2 mRNA expression in peripheral blood mononuclear cells in 11 patients from whom peripheral blood mononuclear cells were

Table 3 Multivariate analysis of predictors for survival

Variable	Hazards ratio	95% CI	P value
Total bilirubin (mg/dL)	1.076	0.484–3.711	0.574
CLIP score (<3)	3.434	0.907–13.000	0.069
Response to therapy (CR or PR)	5.478	1.108–27.093	0.037

Abbreviations: CLIP, Cancer of the Liver Italian Program; CR, complete response; PR, partial response; CI, confidence interval.

Table 4 Univariate and multivariate analyses of predictors for the response to 5-FU + IFN therapy

Variable	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	*P* value	Odds ratio	95% CI	*P* value
Age	1.034	0.951–1.125	0.431	–	–	–
Male	0.333	0.053–2.115	0.244	0.266	0.036–1.966	0.194
HBsAg positive	0.250	0.026–2.416	0.231	0.204	0.019–2.216	0.191
Anti-HCV positive	1.818	0.357–9.272	0.472	–	–	–
Total bilirubin (mg/dL)	0.607	0.132–2.796	0.552	–	–	–
Albumin (g/dL)	1.139	0.206–6.297	0.882	–	–	–
Prothrombin time (%)	0.976	0.912–1.045	0.486	–	–	–
Platelet count ($\times 10^4/\mu L$)	1.012	0.915–1.118	0.820	–	–	–
AFP (<100 ng/mL)	0.880	0.183–4.226	0.873	–	–	–
AFP-L3 (<20%)	0.727	0.151–3.493	0.691	–	–	–
DCP (<100 AU/mL)	0.750	0.067–8.363	0.815	–	–	–
Child–Pugh status A	3.198	0.326–31.39	0.318	–	–	–
Tumor stage III	0.914	0.142–5.902	0.925	–	–	–
JIS score (<3)	0.914	0.142–5.902	0.925	–	–	–
CLIP score (<3)	2.031	0.417–9.886	0.380	–	–	–
Portal vein invasion (<Vp3)	2.031	0.417–9.886	0.380	–	–	–

Abbreviations: HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; NBNC, non-HBV non-HCV; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; TNM, tumor node metastasis; LCSGJ, Liver Cancer Study Group of Japan; JIS, Japan integrated staging; CLIP, Cancer of the Liver Italian Program.

available before therapy, because the effect of 5-FU + IFN has been demonstrated to depend significantly on hepatic IFNAR2 expression,[17] and there is a significant correlation between IFNAR2 expression in the liver and peripheral blood mononuclear cells.[26] Seven of the 11 patients were responders (complete response/partial response) and the remaining four patients were nonresponders (stable disease/progressive disease). The expression level of IFNAR2 in peripheral blood mononuclear cells was significantly ($P = 0.012$) higher in responders (6.5 ± 2.4) than in nonresponders (2.4 ± 0.6, see Figure 2).

Adverse reactions and complications

Most patients complained of flu-like symptoms, including fever, nausea, and loss of appetite, but the degree of these adverse reactions was NCI-CTC Grade 1 or 2. Among patients with NCI-CTC Grade 3 adverse reactions, stomatitis was observed in two patients, diarrhea in one, leukopenia in one, thrombocytopenia in one, and hemorrhagic gastric ulcer in another. None of the patients required administration of granulocyte colony-stimulating factor or blood transfusion. There were five complications resulting from the arterial catheter, ie, occlusion in two patients, infection associated with the indwelling catheter in two patients, and dislocation in a further patient.

Additional therapy

Three patients each were treated with transcatheter arterial chemoembolization and intra-arterial 5-FU + cisplatin, respectively, after identification of progressive disease. Three patients assessed to exhibit a partial response had additional therapy, one of whom underwent partial hepatectomy because of downstaging of hepatocellular carcinoma, and the other two were repeatedly treated with transcatheter arterial chemoembolization because of dislocation of an indwelling intra-arterial catheter or downstaging of

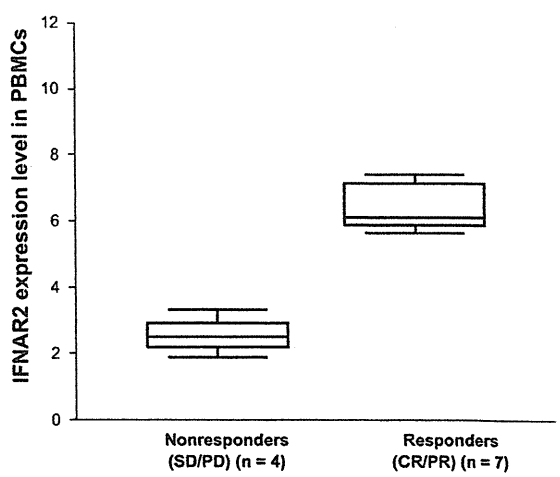

Figure 2 Expression levels of IFNAR2 in peripheral blood mononuclear cells in responders (complete response or partial response) and nonresponders (stable disease or progressive disease) to 5-FU + IFN therapy. The relative quantities of IFNAR2 mRNA in peripheral blood mononuclear cells were normalized to β-actin mRNA. The results are shown as box plot profiles. The bottom and top edges of the boxes are the 25th and 75th percentiles, respectively. Median values are shown by the lines within the boxes. IFNAR2 expression was significantly higher in responders than in nonresponders (Mann–Whitney *U* test, $P = 0.012$).
Abbreviations: IFNAR2, Type I interferon alpha receptor 2; 5-FU, 5-fluorouracil; IFN, interferon.

hepatocellular carcinoma. Twenty-one patients, including the complete responder, did not have additional therapy.

Discussion

The response rate (30%), median survival (7.5 months), and one-year and two-year cumulative survival rates (53% and 33%) for patients in this study were comparable with those reported for previous studies.[17–19] Although the median survival time of nonresponders was identical to that of all patients (7.5 months), the mean survival time of nonresponders (343 ± 272 days) was shorter than that for all patients (505 ± 574 days). This may be explained by the fact that some responders with advanced hepatocellular carcinoma showed considerably long survival, as shown in the Kaplan–Meier survival curve. One complete responder (2402 days) and one partial responder (1957 days) who underwent partial hepatectomy as additional therapy remain alive without recurrence of hepatocellular carcinoma. Two partial responders treated with transcatheter arterial chemoembolization as additional therapy showed long survival (1326 days and 1280 days). Thus, adequate additional therapy preceded by downstaging of hepatocellular carcinoma in response to the 5-FU + IFN combination may be important for responders to obtain long survival.

Several possible mechanisms for the anticancer effects of 5-FU + IFN therapy have been proposed. Transcription of the tumor suppressor p53 gene has been demonstrated to be induced by IFN-α/β, accompanied by an increase in p53 protein levels, suggesting the integration of IFN-α/β signaling into p53 responses in tumor suppression.[27] Yamamoto et al reported that the tumor necrosis factor-related apoptosis-inducing ligand receptor-mediated cytotoxic pathway could be involved in the antihepatocellular carcinoma effect of the 5-FU + IFN combination.[28] It is also possible that IFN and 5-FU reinforce the antitumor action of each other or have additive effects. The cytotoxic effect of 5-FU enhanced by IFN in various cultured malignant cells and upregulation of 5-FU activity when combined with IFN has been demonstrated.[29–31]

Response to therapy was the sole significant and independent predictor for survival of patients with advanced hepatocellular carcinoma who received the 5-FU + IFN combination in the present study. It should be noted that identification of response to therapy (complete response or partial response) as a predictor for survival was common to three Japanese studies,[17–19] in addition to our study, despite different patient populations based on different grades of portal venous invasion and/or different evaluations of responses to therapy (RECIST or ECOG criteria). These results suggest that the response to therapy (complete response or partial response) is indeed critical for patients with advanced hepatocellular carcinoma who receive the 5-FU + IFN combination to have better survival.

Although previous studies have demonstrated several predictors of survival other than response to therapy, such as positivity for anti-HCV antibodies, performance status, and/or total bilirubin level,[18,19] this discrepancy may be explained by the different patient populations in the relevant studies as a result of the different eligibility criteria used. In contrast, use of the same criteria for evaluation of response to therapy (RECIST), in addition to similar patient populations, showed almost the same objective response rates (complete response and partial response patients/all patients) in our study (30%) and that of Uka et al (29%).[19] Despite the prominent improvement in survival of responders (complete response or partial response), it must be acknowledged that the response rates were not satisfactory, suggesting that more than half of patients with advanced hepatocellular carcinoma would remain unresponsive to the 5-FU + IFN combination. We also have to consider that this combination therapy has a considerable negative impact on quality of life for patients with advanced hepatocellular carcinoma, even though adverse reactions were rarely severe in the present study. Therefore, it appears to be very important to predict responders beforehand in the clinical setting.

Ota et al demonstrated that, among several clinical parameters, including α-fetoprotein, des-γ-carboxy prothrombin, Child–Pugh score, and CLIP score, the hepatic expression of IFNAR2 was the only significant predictor of clinical response to 5-FU + IFN therapy.[17] It is particularly noteworthy that all patients without IFNAR2 expression in hepatocellular carcinoma tissue are not responsive to 5-FU + IFN therapy. The importance of IFNAR2 expression for the anticancer effect of 5-FU + IFN has also been shown by in vitro analysis.[32,33] IFNAR2 expression in hepatocellular carcinoma tissue was assessed immunohistochemically at the protein level in the study by Ota et al. We have previously shown a correlation between IFNAR2 protein expression and IFNAR2 mRNA expression in liver specimens from patients with chronic hepatitis C.[34] We have also found a correlation between IFNAR2 mRNA expression in peripheral blood mononuclear cells and in the livers of patients with chronic hepatitis C.[26] Therefore, IFNAR2 expression in hepatocellular carcinoma tissue is likely to be correlated with that in

peripheral blood mononuclear cells, even though there have been no reports explaining the possible mechanisms for this correlation, as far as we know. Liver biopsy is sometimes difficult to perform before combination therapy in patients with advanced hepatocellular carcinoma because of the bleeding tendency arising from a low count platelet and/or decreased activity of prothrombin. IFNAR2 mRNA expression in peripheral blood mononuclear cells was significantly higher in responders (complete response or partial response) than in nonresponders (stable disease or progressive disease) in the present study. Based on these results, we propose a testable hypothesis that IFNAR2 expression in peripheral blood mononuclear cells may be a practical predictor of response to the 5-FU + IFN combination.

Several limitations existed in this study. First, a significant percentage of patients who fulfilled the eligibility criteria could not be included due to lack of written informed consent. Second, the number of patients in whom IFNAR2 expression was examined for peripheral blood mononuclear cells was too small to draw a definitive conclusion. We could not evaluate if IFNAR2 expression in peripheral blood mononuclear cells could be an independent predictor for response to the 5-FU + IFN combination in multivariate analysis. Further studies need to be conducted in a larger number of patients to clarify the clinical usefulness of measurement of IFNAR2 expression in peripheral blood mononuclear cells as a predictor of response to the 5-FU + IFN combination. Third, the correlation between IFNAR2 protein expression and IFNAR2 mRNA expression in peripheral blood mononuclear cells was not examined, even though we have previously confirmed this correlation in the liver.

In conclusion, we have shown preliminary evidence that IFNAR2 expression in peripheral blood mononuclear cells may predict the response to 5-FU + IFN therapy beforehand in patients with advanced hepatocellular carcinoma, which should enable us to treat those patients who are likely to respond to this combination therapy in a selective manner.

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Disclosure

The authors report no conflicts of interest in this work.

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□ CASE REPORT □

Focal Nodular Hyperplasia-Like Nodule with Reduced Expression of Organic Anion Transporter 1B3 in Alcoholic Liver Cirrhosis

Nobuko Doi[1], Yasuyuki Tomiyama[1], Tomoya Kawase [1], Sohji Nishina[1], Naoko Yoshioka[1], Yuichi Hara[1], Koji Yoshida[1], Keiko Korenaga[1], Masaaki Korenaga[1], Takuya Moriya[2], Atsushi Urakami[3], Osamu Nakashima[4], Masamichi Kojiro[4] and Keisuke Hino[1]

Abstract

We report a patient with alcoholic liver cirrhosis who had a 15 mm focal nodular hyperplasia (FNH)-like nodule in the liver. This FNH-like nodule was diagnosed as hepatocellular carcinoma (HCC) mainly based on hypervascularity during the hepatic arterial phase, washout pattern during the equilibrium phase and low signal intensity during the hepatobiliary phase in gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced MRI; it was surgically resected. Its histology exhibited hepatocyte hyperplasia, fibrous septa containing unpaired small arteries accompanied by reactive bile ductules, remarkable iron deposits and sinusoidal capillarization, and was compatible with the diagnosis of an FNH-like nodule. When we analyzed the images of the present nodule retrospectively, low signal intensity on in-phase and isosignal intensity on opposed-phase T1-weighted MRI may have reflected iron deposits in the FNH-like nodule. In addition, a low signal intensity on T2-weighted MRI and no detection in diffusion-weighted MRI may help in distinguishing FNH-like nodules from HCC, since these image findings are inconsistent with typical HCC. Immunohistochemical analysis revealed a markedly reduced expression of organic anion transporter (OATP) 1B3 in this nodule, which implied decreased Gd-EOB-DTPA uptake by hepatocytes and accounted for the low signal intensity during the hepatobiliary phase on Gd-EOB-DTPA-enhanced MRI. To the best of our knowledge this is the first report in which an FNH-like nodule was assessed for OATP1B3 expression.

Key words: alcoholic liver cirrhosis, FNH-like nodule, hepatocellular carcinoma, organic anion transporter, Gd-EOB-DTPA-enhanced MRI

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Introduction

Due to improvements in imaging techniques and pathological evaluation, a new type of small focal lesion occurring in the cirrhotic liver has been described (1-3). Focal nodular hyperplasia (FNH)-like nodules (FNH-like nodules) are focal lesions occurring in liver cirrhosis and are morphologically very similar to classical FNH in the otherwise normal liver. In general, FNH-like nodules are assumed not to have an increased risk of malignant transformation (1-3), but this issue remains elusive (4). FNH-like nodules are occasionally misdiagnosed on imaging as hepatocellular carcinoma (HCC) due to hypervascularity during the arterial phase of magnetic resonance imaging (MRI)/computed tomography (CT).

On the other hand, gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-

[1]Departments of Hepatology and Pancreatology, Kawasaki Medical University, Japan, [2]Department of Pathology, Kawasaki Medical University, Japan, [3]Department of Digestive Surgery, Kawasaki Medical University, Japan and [4]Department of Pathology, Kurume University School of Medicine, Japan
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Correspondence to Dr. Keisuke Hino, khino@med.kawasaki-m.ac.jp

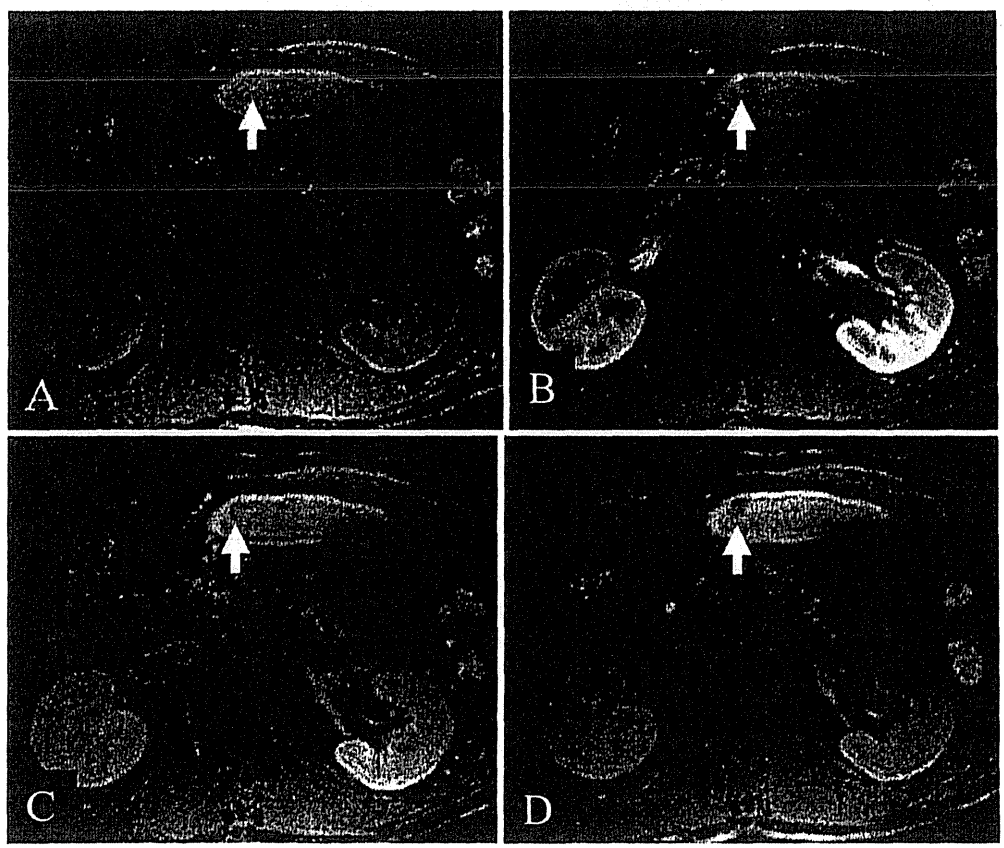

Figure 1. Images of the FNH-like nodule in segment 3 in Gd-EOB-DTPA-enhanced MRI. Arrows indicate a 9mm FNH-like nodule. (A) Low signal intensity before contrast injection, (B) High signal intensity during the hepatic arterial phase, (C) Washout pattern during the equilibrium phase, (D) Low signal intensity during the hepatobiliary phase.

enhanced MRI has enabled us to detect focal liver lesions because of its hepatocyte-specific properties (5-7), and it might be the most useful imaging modality for the diagnosis of HCC at present (8, 9). However, the image findings of FNH-like nodules in Gd-EOB-DTPA-enhanced MRI are not well known, and it remains unclear if FNH-like nodules can be distinguished from HCC in Gd-EOB-DTPA-enhanced MRI. Here, we report a histologically proven FNH-like nodule in a patient with alcoholic liver cirrhosis, and discuss the diagnostic potential of Gd-EOB-DTPA-enhanced MRI for FNH-like nodules.

Case Report

A 68-year-old Japanese man with a history of alcoholic liver cirrhosis for approximately 10 years was found to have a 9 mm hypervascular nodule in the liver through contrast-enhanced CT and admitted to Kawasaki Medical University Hospital in June 2008 for further examination of the hepatic nodule.

His alcoholic consumption over the previous 40 years was 100 g or more per day. A physical examination on admission showed no remarkable abnormalities except for moderate splenomegaly. Laboratory data on admission disclosed the following abnormal values: platelet count $9.4\times10^4/\mu L$ (normal range 15-35), aspartate aminotransferase 58 IU/L (10-35), γ-glutamyl transpeptidase 346 IU/L (5-60) and indocyanine green retention rate at 15 minutes 16.4% (<10). The levels of hepatic tumor markers were as follows: α-fetoprotein 9.0 ng/mL (<10) and des-γ-carboxy prothrombin 25 mAU/mL (<40). The serum was negative for anti-hepatitis C virus antibody and hepatitis B surface (HBs) antigen but positive for anti-HBs and anti-hepatitis B core antibodies.

Neither B-mode sonographic scans nor Sonazoid contrast-enhanced ultrasonography detected the hepatic nodule. Arteriography did not disclose any hypervascular mass lesion. Contrast-enhanced CT revealed a nodule of 9 mm in the liver segment 3 as hypervascularity during the hepatic arterial phase. Gd-EOB-DTPA-enhanced MRI disclosed that this nodule had a low signal intensity before contrast injection (Fig. 1A), hypervascularity during the hepatic arterial phase (Fig. 1B), a washout pattern during the equilibrium phase (Fig. 1C), and a low signal intensity during the hepatobiliary phase (Fig. 1D). Diffusion-weighted MRI did not reveal this nodule (Fig. 2A). In- and opposed-phase T1-weighted MRI, and T2-weighted MRI disclosed this nodule as low signal intensity (Fig. 2B), isosignal intensity (Fig. 2C) and slightly

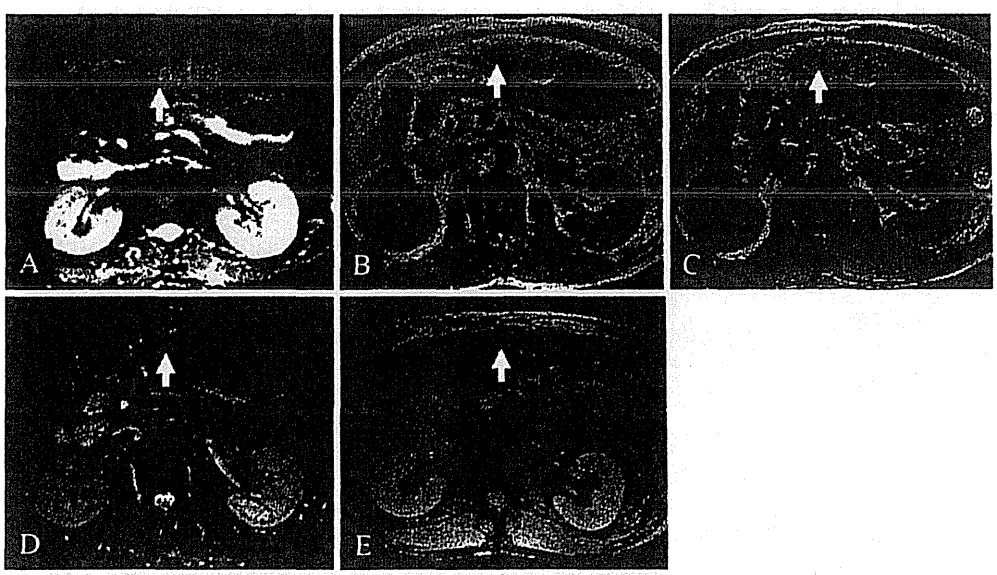

Figure 2. Images of the FNH-like nodule in segment 3 in Gd-EOB-DTPA-enhanced MRI. Arrows indicate the 9mm FNH-like nodule. (A) No detection of nodule in diffusion-weighted MRI, (B) Low signal intensity on in-phase T1-weighted MRI, (C) Isosignal intensity on opposed-phase T1-weighted MRI, (D) Slightly low signal intensity in T2-weighted MRI, (E) Slightly low signal intensity in SPIO-enhanced MRI.

low signal intensity (Fig. 2D), respectively. Although this nodule was detected as slightly low signal intensity (Fig. 2E) in superparamagnetic iron oxide (SPIO)-enhanced MRI, it was uncertain if Kupffer cells took up SPIO because of the slightly low signal intensity on T2-weighted MRI before SPIO injection.

The imaging findings mentioned above were suggestive of HCC, even though several findings, such as low signal intensity on in-phase and isosignal intensity on opposed-phase T1-weighted MRI, low signal intensity in T2-weighted MRI and no detection in diffusion-weighted MRI, were not consistent with typical HCC. We could not histologically assess this hepatic nodule by liver biopsy because of its undetectability by ultrasonography, and we could not ignore the possibility of HCC as the diagnosis of this nodule. Therefore, this nodule was surgically resected after obtaining informed consent from the patient. The nodule of interest was not encapsulated and its margin was difficult to distinguish from the surrounding cirrhotic tissue (Fig. 3A and 3B). Intranodular fibrous septa were present but central fibrous scarring and portal tracts were absent (Fig. 3C). The fibrous septa contained unpaired small arteries accompanied by reactive bile ductules radiating into the parenchyma (Fig. 3D). This nodule showed varying degrees of increased cellularity (Fig. 4A) and marked iron deposits in the hepatocyte and/or Kupffer cells (Fig. 4B) compared to the surrounding cirrhotic tissue. Immunohistochemical analysis using an anti-CD34 antibody (anti-CD34) revealed marked sinusoidal capillarization (Fig. 4C). Thus, the histological diagnosis of this nodule was an FNH-like nodule. Finally, we immunohistochemically assessed the expression of organic anion transporter (OATP) 1B3 in hepatocytes, using an anti-OATP1B3 antibody (anti-OATP1B3) to examine why this nodule exhibited low signal intensity during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI. Immunohistochemically, OATP1B3 was diffusely and strongly positive for the cell membrane of the hepatocytes in the surrounding cirrhotic tissue, but was nearly absent in the FNH-like nodule (Fig. 5A-C). Thus far neither recurrence of the FNH-like nodule nor the development of HCC has been found in this patient who has stopped drinking alcohol since he was admitted to our hospital.

Discussion

FNH-like nodules occurring in cirrhotic livers are reported to have the pathological features such as encapsulation, hepatocyte hyperplasia, fibrous septa containing unpaired small arteries accompanied by reactive bile ductules, iron deposits and/or sinusoidal capillarization (1, 2). It has been suggested that the artery-dominant condition derived from disturbed portal circulation in the cirrhotic liver (10) or the congenital vascular anomaly (11, 12) causes localized hyperplastic changes of the hepatocytes, and generates nodular lesions such as FNH. The increased unpaired arteries, diffuse capillarization, and iron deposits in the nodule would be attributable to a similar mechanism in nodular formation. The FNH-like nodule in this study had these pathological features except for encapsulation. One possible explanation for the lack of encapsulation is that hepatocytic hyperplasia had not expanded sufficiently to be encapsulated because it was the early stage in the development of the hyperplastic nod-

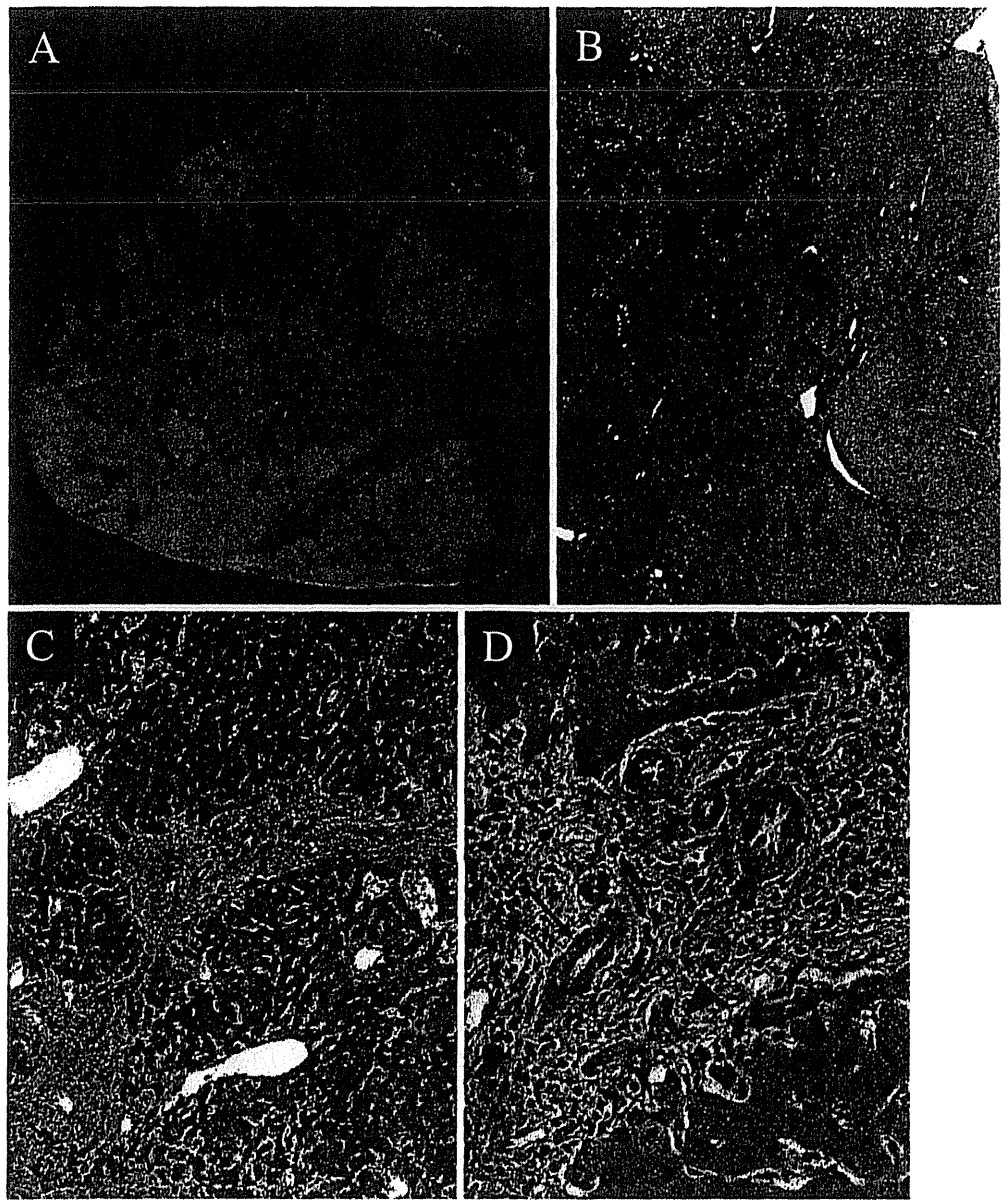

Figure 3. Surgically resected specimen and histology of the FNH-like nodule. (A) Arrows indicate the FNH-like nodule (15mm). The nodule is not encapsulated and its margin is difficult to distinguish from the surrounding tissue. (B) The surrounding tissue shows liver cirrhosis (Masson trichrome ×40). (C) Fibrovascular septa with mild lymphocyte infiltrate within the FNH-like nodule (Hematoxylin and Eosin staining ×100), (D) Unpaired small arteries (arrows) and reactive bile ductules radiating into the parenchyma (arrowheads) within a fibrovascular septum in the FNH-like nodule (Hematoxylin and Eosin staining ×400).

ule. In this respect the state of the present FNH-like nodule may suggest its early stage. The present case clearly indicated the existence of an FNH-like nodule with reduced OATP1B3 expression. Hepatocytic disorder derived from disturbed portal circulation in cirrhotic liver may have suppressed the expression of OATP1B3. We cannot necessarily exclude a possibility of malignant potential of this nodule in terms of nearly absent expression of OATP1B3. Otherwise, unknown mechanisms may have been related to the reduced expression of OATP1B3.

FNH-like nodules also are clinically important lesions in terms of difficulty in distinguishing them from well-differentiated HCC in image diagnosis. There were at least two reasons why we had diagnosed this patient as having probable HCC in imaging. First, the present FNH-like nodule exhibited hypervascularity during the hepatic arterial phase and a washout pattern during the equilibrium phase in contrast-enhanced MRI. Second, the Gd-EOB-DTPA-enhanced MRI revealed this nodule to have low signal intensity during the hepatobiliary phase, which implied re-

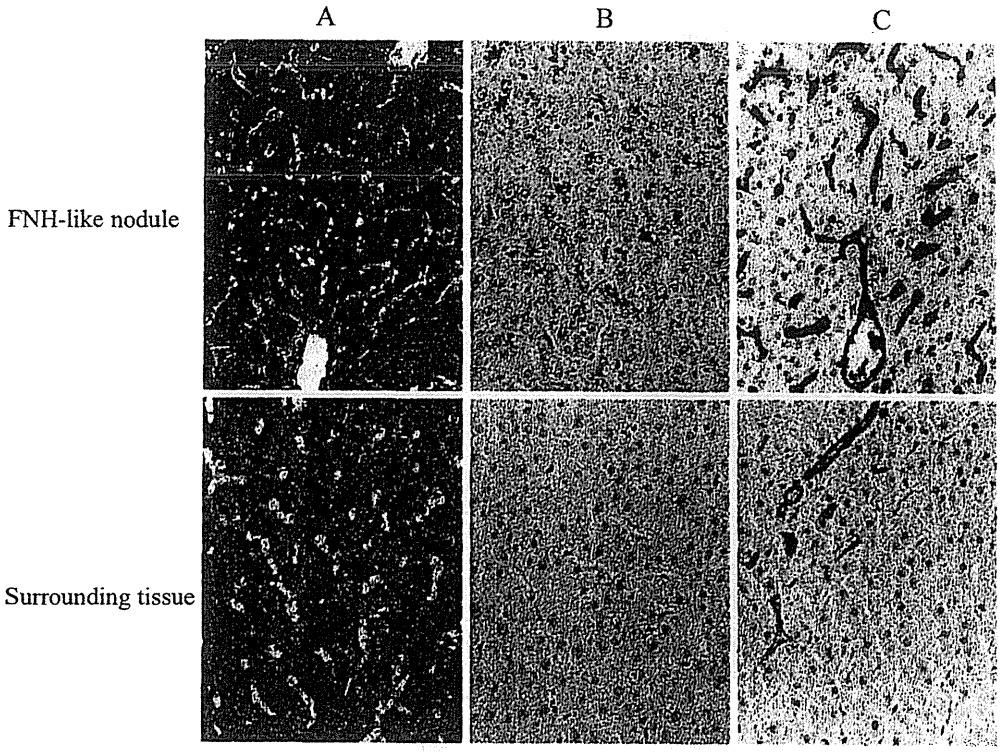

Figure 4. Cell density, iron deposits and sinusoidal capillarization in the FNH-like nodule and the surrounding tissue. The FNH-like nodule shows increased cell density (A, Hematoxylin and Eosin staining ×400), remarkable iron deposits in the hepatocyte and/or Kupffer cells (B, Berlin blue ×400) and marked sinusoidal capillarization (C, immunohistochemical staining using anti-CD34 ×400), compared to the surrounding tissue.

duced uptake of Gd-EOB-DTPA by hepatocytes. Reduced Gd-EOB-DTPA uptake by hepatocytes was reported to suggest an early event of hepatocarcinogenesis in a recent study (13). In contrast, FNH is demonstrated to be enhanced during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (5, 14). With respect to this point, it should be noted that the present FNH-like nodule may have had an exceptionally low signal intensity during the hepatobiliary phase. The present results were consistent with the recent report that uptake of Gd-EOB-DTPA is determined by OATP1B3 expression rather than by tumor differentiation or bile production in HCC (15), and suggested the difficulty in discriminating between FNH-like nodules and HCC by assessing the Gd-EOB-DTPA uptake by hepatocytes.

Which MRI imaging findings were useful for distinguishing between FNH-like nodules and HCC in this patient? When we analyzed the images of this nodule retrospectively, there seemed to be three important findings for diagnosis. First, the low signal intensity on in-phase and isosignal intensity on opposed-phase T1-weighted MRI may have reflected iron deposits in the FNH-like nodule, since similar phase-shift imaging has been reported to reflect hemosiderin deposits in regenerative nodules in liver cirrhosis (16). In contrast, the isointensity to slightly high intensity on in-phase and the low signal intensity on opposed-phase T1-weighted MRI are known to reflect hepatocellular nodules with fatty degeneration (8). Thus, the combined findings from the in-phase and opposed-phase may facilitate discrimination between FNH-like nodules and well-differentiated HCC, since the former frequently have iron deposits and the latter has fatty degeneration. Second, FNH-like nodules and HCC have been shown to be likely to exhibit iso- to low signal intensity and high signal intensity in T2-weighted MRI, respectively (17), which was consistent with the low signal intensity in the present nodule. Third, the lack of detection in diffusion-weighted MRI may help in distinguishing FNH-like nodules from HCC, since diffusion-weighted MRI imaging has been reported to be useful in differentiating benign hepatocellular nodules including FNH from HCC (18). However, it still may be difficult to distinguish such small FNH-like nodules showing low signal intensity during the hepatobiliary phase in Gd-EOB-DTPA-enhanced MRI from HCC in clinical practice.

In addition, it remains controversial whether FNH-like nodules can be distinguished from HCC based on the presence of Kupffer cells in the nodules. A defect in the Kupffer phase on contrast-enhanced ultrasonography, which implies the absence of Kupffer cells, has been reported in the FNH-like nodule in alcoholic liver cirrhosis (19), whereas the presence of Kupffer cells on SPIO-enhanced MRI has also been shown in FNH-like nodules in alcoholic liver cirrhosis (17). The present FNH-like nodule may have contained

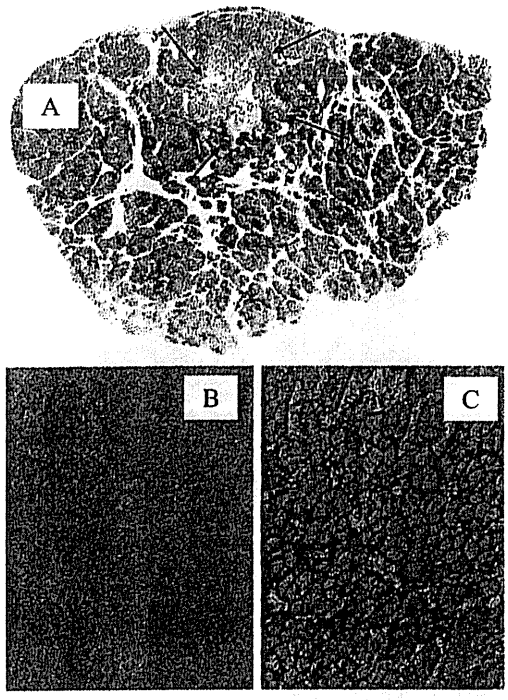

Figure 5. Expression of OATP1B3 in surgically resected specimen. Arrows indicate the FNH-like nodule (A). The expression of OATP1B3 is nearly absent in the nodule (B, ×400), but is diffusely found in the surrounding tissue (C, ×400). OATP1B3 was immunohistochemically detected using anti-OATP1B3.

Kupffer cells, since Sonazoid contrast-enhanced ultrasonography did not detect this nodule. However, we could not precisely assess the uptake of SPIO by Kupffer cells because of the slightly low signal intensity on T2-weighted MRI before SPIO injection. Thus, the present case suggests the importance of pathological diagnosis for hepatic small nodular lesions as well as the difficulty in image diagnosis for such lesions. We also propose that observational follow-up is also an important modality to be chosen when nodules are less than 1.5 cm in diameter, since small nodular lesions associated with chronic liver diseases smaller than 1.5 cm have been reported to have less potential to be early HCC (20).

In conclusion, we found an FNH-like nodule with reduced expression of OATP1B3 in a patient with alcoholic liver cirrhosis, and retrospectively analyzed imaging findings useful for distinguishing FNH-like nodules from HCC.

The authors state that they have no Conflict of Interest (COI).

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肝炎ウイルスによる発癌のメカニズム

仁科惣治・是永匡紹・日野啓輔

東京 文光堂 本郷

肝炎ウイルスによる発癌のメカニズム

仁科惣治・是永匡紹・日野啓輔
川崎医科大学肝胆膵内科学／にしな・そおじ　これなが・まさあき　ひの・けいすけ

はじめに

肝細胞癌は原発性肝癌の約8～9割を占める．わが国における原発性肝細胞癌の特徴は，そのほとんどがB型肝炎ウイルス(HBV)，あるいはC型肝炎ウイルス(HCV)の持続感染を有していることである．そこで本稿では，これらの肝炎ウイルスによる発癌メカニズムについての最近の知見を紹介する．

HBV と肝発癌

臨床的にもHBV-DNA量に依存した肝発癌の報告[1]やときに肝線維化の進行していないほぼ正常肝から肝発癌を認めることからもHBVによる直接的な肝発癌機構が想像されるが，まだその機序については不明な点が多い．ここでは実験的なevidenceを基にしたいくつかの肝発癌機序を紹介する．

1. HBV 関連蛋白による直接作用

炎症反応を伴わない*HBx*遺伝子導入マウスでは肝発癌を認めたが[2]，その理由として*HBx*遺伝子産物であるX蛋白が① 転写因子を介して増殖に関連する遺伝子を活性化すること，② 細胞周期の制御機構を障害すること，③ 癌抑制遺伝子*p53*に結合してDNA修復機能を抑制することなどがあげられている[3]．また，HBVの表面蛋白(S蛋白)をコードする*PreS2*遺伝子を導入したマウスでも高頻度に肝発癌を認めたとの報告がある[4]．その理由として，PreS2蛋白質が細胞増殖に関連するシグナル伝達分子であるMAPK(mitogen-activated protein kinase)を活性化し細胞増殖を起こすことなどが考えられる．

2. 宿主 DNA への HBV-DNA の組み込み

また宿主DNAへのHBV-DNAの組み込みintegrationも肝発癌の原因として重要であるが，HBV関連肝細胞癌では約90％の症例で認められる．HBVは複製過程で逆転写反応を介してpregenomic RNAからウイルスDNAを合成するが，このときに直鎖状二本鎖HBV-DNAが宿主DNAへ組み込まれる．組み込まれるHBV遺伝子としては*X*遺伝子や，部分欠損した形での*preS2/S*遺伝子といわれている[5]．

3. 肝発癌に対するアンドロゲン濃度やアンドロゲン受容体(AR)の関与

HCV関連肝発癌では男性が女性の2～3倍であるのに対し，HBV関連肝発癌では5～7倍と高いことが報告されている[6,7]．最近，こうした肝発癌における性差を説明するメカニズムもいくつか報告されている．HBV関連肝発癌とは直接的に関連しないものの，マウスのdiethylnitrosamine(DEN)化学肝発癌においてエストロゲンがKupffer細胞からのIL-6分泌を抑制することで肝発癌を抑制することが報告された[8]．またmicro RNA(miRNA)-18aによるエストロゲン受容体(ER)αの翻訳阻害と肝細胞増殖による肝発癌機構[9]などが報告されている．一方，HBV関連肝発癌との関連として，HBx蛋白存在下でのアンドロゲン受容体転写能活性亢進による肝発癌機構[10]やアンドロゲン受容体によるHBV-RNA転写亢進がHBV量を増加し，協同的に増加するHBx蛋白とともに肝発癌を促進する[11]ことが報告されている．

HCV と肝発癌

1. HCV の遺伝子構造

HCVはプラス鎖RNAウイルスであり，約9,500ヌクレオチド長の一本鎖RNAをそのゲノムとしている．そのゲノムの大部分は一つの蛋白質読み枠openreading frame(ORF)で占められ，ウイルス蛋白はすべてここにコードされている．DNAウイルスなどの他の癌ウイルスの場合ではウイルスゲノムは宿主細胞の核内で複製され，そ

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●HBV 関連肝発癌は血清 HBV-DNA 量と正の相関関係がある.
●HBV 関連肝発癌の機序として，X 蛋白や PreS2 蛋白，宿主 DNA への HBV-DNA 組み込み，アンドロゲン受容体が関与する説がある.
●HCV 関連肝発癌には HCV コア蛋白によるミトコンドリア障害を介した活性酸素が関与している.

のゲノムが宿主染色体に挿入され，それが発癌の原因となる場合がある．しかし HCV ゲノムは細胞質において複製すると考えられており，現在のところ HCV-RNA あるいは cDNA は核内から検出されていない．このことからその遺伝子産物つまり HCV 蛋白が細胞の癌化に対して何らかの機能を持つと考えられているわけである.

2. HCV と酸化ストレス

HCV 構造蛋白の一つである HCV コア蛋白は *in vivo* や *in vitro* の系で酸化ストレスを誘導することが明らかにされている．HCV コア遺伝子導入マウスでの還元型グルタチオン(GSH)の低下や加齢に伴う酸化ストレス亢進が報告されている[12]．また HCV コア蛋白発現培養細胞でも活性酸素や過酸化脂質が上昇し，抗酸化反応因子の遺伝子発現が誘導されることから，酸化ストレスが HCV コア蛋白により直接に引き起こされていると考えられた[13]．ミトコンドリアは細胞内の活性酸素の最大産生部位であるが，われわれや他の研究者により HCV コア蛋白が呼吸鎖複合体 I の機能障害を引き起こす[14]ことや，ミトコンドリアシャペロン蛋白である prohibitin と cytochrome oxidase (COX)の相互作用が抑制され，COX の活性低下を引き起こす[15]ことが報告されている．このように HCV コア蛋白による活性酸素発生部位としてミトコンドリアが重要であることが明らかとなり，ミトコンドリア障害に基づく酸化ストレスが肝発癌を引き起こすことがいくつかの HCV トランスジェニックマウスの系で明らかにされている[16, 17]．さらに，HCV 蛋白は酸化ストレス刺激に対する感受性を亢進させて肝発癌を引き起こし，例えばわれわれは HCV トランスジェニックマウスに微量の鉄負荷を行うことでミトコンドリア障害，酸化的 DNA 障害が亢進して肝発癌が促進されることを明らかにした[18] (図 1).

3. HCV による epigenetic な遺伝子変異

a. retinoblastoma 癌抑制蛋白(Rb)

Rb はアデノウイルス，ヒトパピローマウイルスのような DNA 癌ウイルスが発現する癌蛋白の共通した標的となる．Rb を抑制すると細胞周期開始が促進され，これらのウイルスの増殖に必要な DNA 合成経路が促進される．しかし驚くべきことに Rb は HCV のような RNA ウイルスの標的にもなっており，NS5B は細胞質内で Rb と複合体を形成し Rb に E6 関連蛋白(E6AP)が結合することでポリユビキチン化が進みプロテアーゼによる Rb 変性が起こる．その結果，細胞周期の S 期へのエントリーを促進するとされている E2F 反応性プロモーターが活性化される[19].

b. *p53* 経路

コアを含めた HCV 蛋白と *p53* との相互関係は実験系により controversial ではあるものの，NS3 や NS5A 蛋白が *p53* と相互作用し *p53* 依存性の転写を抑制し得ることが報告されている．また，NS5B 蛋白が *p53* の転写促進因子であり RNA helicase のひとつである DDX5 と相互作用してその機能を阻害することも報告されている[19]．こうした HCV 蛋白による *p53* の阻害機能は HCV 関連肝発癌の一機序と考えられるが，さらなるデータの集積が必要である.

c. Wnt/β-カテニン

Wnt 経路の構成要素は肝癌でしばしば変異し，その結果 β-カテニンを安定化させる．これにより β-カテニンは核内に移行することができ，細胞増殖に影響する遺伝子の転写を調節する蛋白と相互作用することができる．ここでは詳細な機序は割愛するが，NS5A 蛋白が β-カテニンを安定化させることで，β-カテニン依存的な転写活性が亢進することも報告されており，HCV 関連肝発癌との関連を考えるうえで興味深い[19]