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.

Acknowledgments

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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

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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)-

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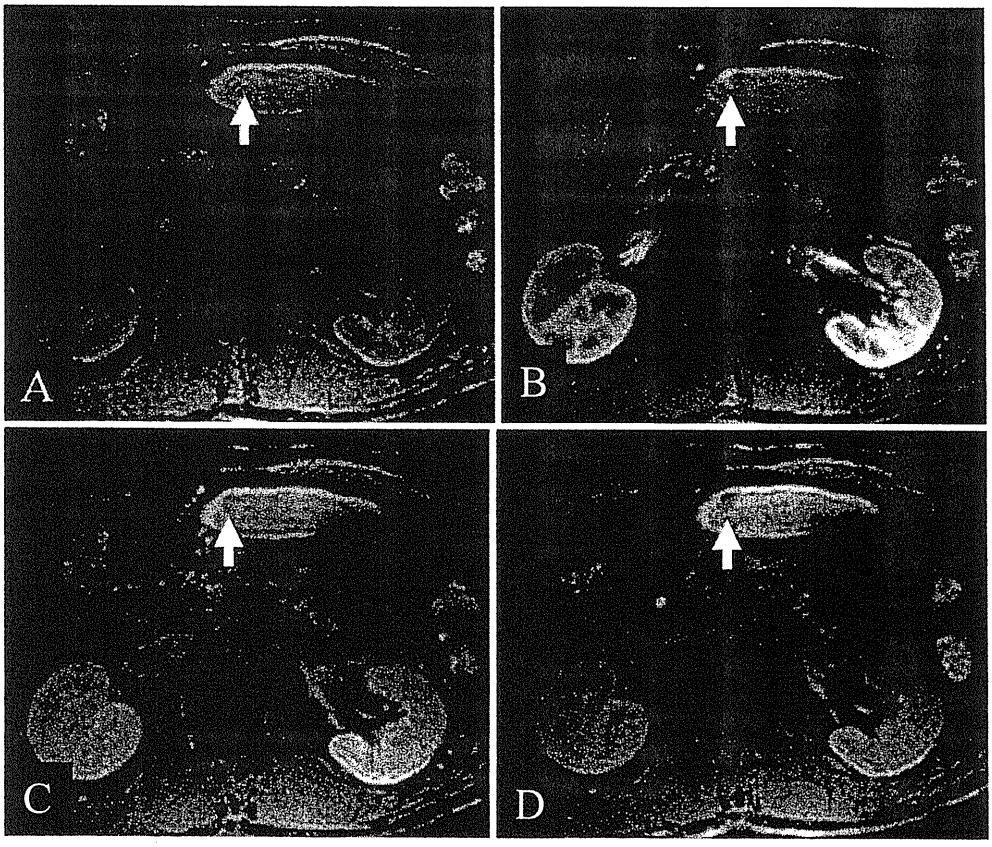

Figure 1. Images of the FNH-like nodule in segment 3 in Gd-EOB-DTPA-enhanced MRI. Arrows indicate a 9mm FNH-like nodule. (A) Low signal intensity before contrast injection, (B) High signal intensity during the hepatic arterial phase, (C) Washout pattern during the equilibrium phase, (D) Low signal intensity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

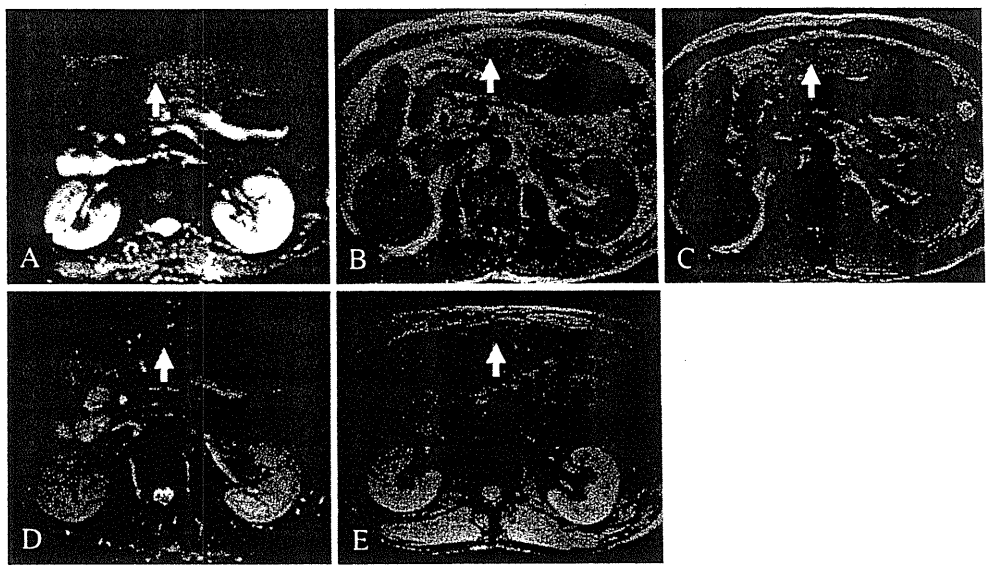

Figure 2. Images of the FNH-like nodule in segment 3 in Gd-EOB-DTPA-enhanced MRI. Arrows indicate the 9mm FNH-like nodule. (A) No detection of nodule in diffusion-weighted MRI, (B) Low signal intensity on in-phase T1-weighted MRI, (C) Isosignal intensity on opposed-phase T1-weighted MRI, (D) Slightly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

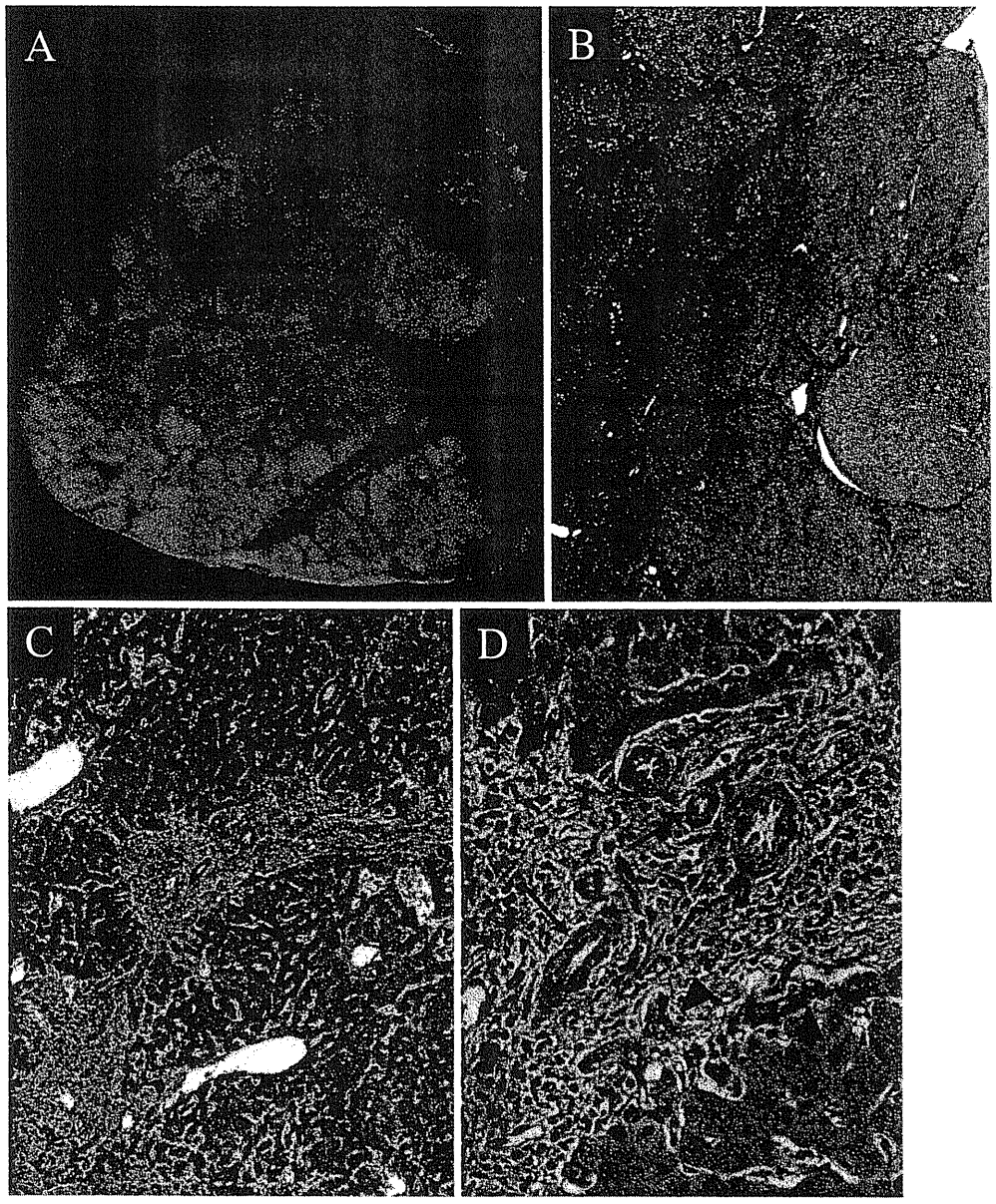

Figure 3. Surgically resected specimen and histology of the FNH-like nodule. (A) Arrows indicate the FNH-like nodule (15mm). The nodule is not encapsulated and its margin is difficult to distinguish from the surrounding tissue. (B) The surrounding tissue shows liver cirrhosis (Masson trichrome ×40). (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-

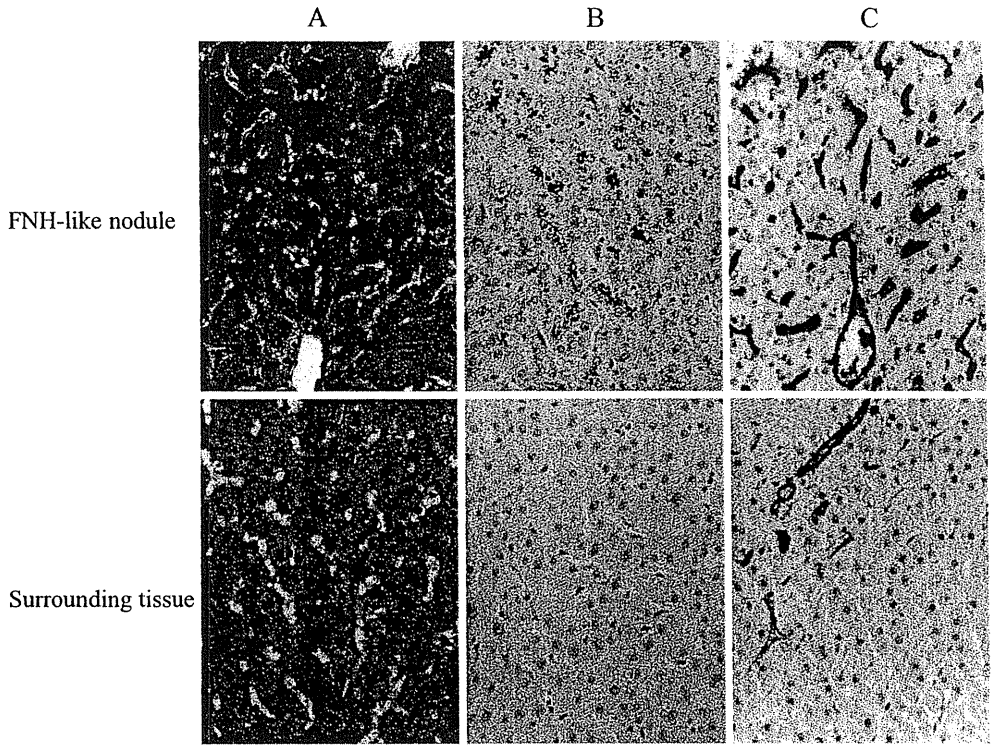

Figure 4. Cell density, iron deposits and sinusoidal capillarization in the FNH-like nodule and the surrounding tissue. The FNH-like nodule shows increased cell density (A, Hematoxylin and Eosin staining ×400), remarkable iron deposits in the hepatocyte and/or Kupffer cells (B, Berlin blue ×400) and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

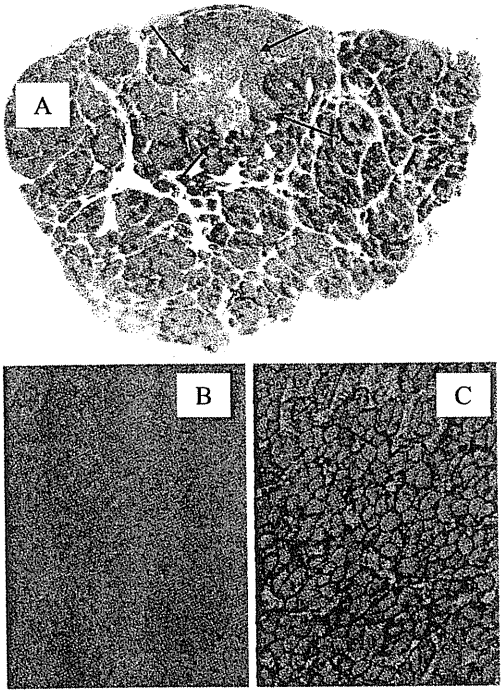

Figure 5. Expression of OATP1B3 in surgically resected specimen. Arrows indicate the FNH-like nodule (A). The expression of OATP1B3 is nearly absent in the nodule (B, ×400), but is diffusely found in the surrounding tissue (C, ×400). OATP1B3 was immunohistochemically detected using anti-OATP1B3.

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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(大阪大学大学院医学系研究科 消化器内科学 教授)

WS2-5　非B非C肝細胞癌，特に成因不明肝細胞癌の臨床的特徴についての検討

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【目的】

近年，非B非C肝細胞癌（以下NBNC-HCC）の割合が増加している．そこでNBNC-HCCの臨床的特徴を明らかにする目的で以下の検討を行った．

【対象・方法】

1999年から2009年の期間で初発のNBNC-HCC46例を対象とした．同時期の初発のHCV抗体陽性肝細胞癌（以下HCV-HCC）で年齢と性差をマッチングさせた46例との比較を行い，NBNC-HCCの臨床的特徴を検討した．また肝切除が施行されたNBNC-HCC11例の背景肝組織を検討し，背景肝の組織からは成因が不明なHCC（以下Cryptogenic-HCC）6例について臨床的特徴を検討した．今回NBNC-HCCの定義はHBs抗原陰性，HCV抗体陰性，アルコール摂取量40g/日未満の症例とし，自己免疫性肝疾患など他の慢性肝疾患からの発癌は除外した．

【結果】

NBNC-HCCはHCV-HCCと比較して糖尿病の合併が多く（P=0.003），BMI（P<0.001），血小板数（P<0.001）が有意に高値であった．また適切な経過観察が行われておらず（P<0.001），

表1　NBNC-HCCとHCV-HCCとの比較

	NBNC-HCC	HCV-HCC	*P* value
Number of patients	46	46	
Age (range)	70± 8.4 (49-84)	70 ± 9.3(49-89)	0.925
Gender (male / female)	37 / 9	33 / 13	0.328
Diabetes Mellitus (＋/－)	26 / 20	12 / 34	0.003 *
BMI	25.8 ± 3.3	22.7 ± 3.1	< 0.001 *
Hypertension (＋/－)	22 / 24	20 / 26	0.675
AFP 陽性 / 陰性	18 / 28	20 / 26	0.672
AFP値	25698.6 ± 151812.5	682.3± 2877.4	0.270
PIVKA II 陽性 / 陰性	38 / 8	31 / 15	0.092
PIVKA II値	22683.1 ± 80344.8	728.9 ± 2083.3	0.070
T-Bil	0.9 ± 0.6	1.0 ± 0.7	0.584
Alb	3.8 ± 0.7	3.8 ± 0.5	0.675
%PT	85.7 ± 15.8	82.3 ± 13.2	0.266
Platelet	16.2 ± 7.0	11.6 ± 4.9	< 0.001 *
ALT	46.2 ± 50.5	57.9 ± 46.8	0.254
AST	57.5 ± 53.4	61.3 ± 38.5	0.701
HBc-Ab (＋/－)	19 / 27	20 / 14	0.121
Child-Pugh A / B	36 / 10	37 / 9	0.797
Stage Ⅰ / Ⅱ / Ⅲ / Ⅳ	4 / 21 / 14 / 7	12 / 25 / 9 / 0	0.006 *
Vp (+/－)	7 / 39	1 / 45	0.029 *
最大腫瘍径 (cm)	4.6 ± 3.5	2.6 ± 1.4	0.001 *
他臓器癌の合併 (＋/－)	13 / 33	6 / 40	0.071
根治療法(肝切除 or 局所治療)/ 非根治療法	27 / 19	33 / 13	0.189
Follow-up （当科 / 他院 / なし）	5 / 0 / 41	9 / 30 / 7	< 0.001 *

* $P < 0.05$

門脈浸潤（P=0.029），最大腫瘍径（P=0.001）に有意差を認め，臨床病期が進行（P=0.006）した状態で診断されるケースが多かった（表1）．肝切除または局所治療による根治療法が施行されたNBNC-HCC(N=27)とHCV-HCC(N=33)において，Kaplan-Meier法を用いて累積生存率を比較したところ両群間に有意差はみられなかったが，無再発生存率はNBNC-HCCが有意に良好であった（図1）．一方，肝切除後に背景肝組織を検討しえたNBNC-HCC患者11例のうち，6例（55%）はF0またはF1と肝線維化に乏しく脂肪化も明らかではない症例，すなわちCryptogenic-HCCであった．組織学的にNASHと診断されたのは1例（9%）のみで，残り4例（36%）はburned out NASHも否定できない肝線維化進展例であった．Cryptogenic-HCC患者6例の平均年齢は65歳，男性が4例，女性が2例，HBc抗体陽性は2例（33.3%）であり，平均腫瘍径は7.4cmであった．AFP陽性は1例（16.7%）のみと陽性率が低い傾向がみられたが，PIVKA IIは5例（83.3%）が陽性であった．また他臓器癌の合併を3例（50%）に認めた．

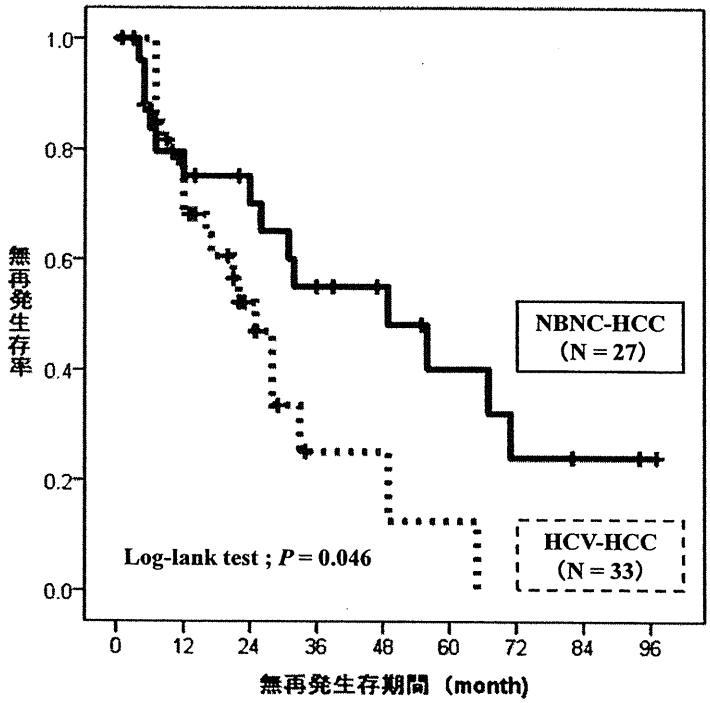

図1　根治療法群における無再発生存率の比較

【まとめ】

NBNC-HCCは糖尿病の合併や肥満例が多く，発癌予防には生活指導の重要性が示唆された．NBNC-HCCでは無再発生存率が有意に良好であり，肝発癌のポテンシャルそのものはHCV-HCCと比較して低いことが予想されるが，累積生存率に差がなかった理由としては二つの可能性が挙げられる．一つは症例数が少なく死亡に至るまでのイベント数が少なかったこと，もう一つは肥満や糖尿病などの生活習慣病や他臓器癌などに起因する他病死が生存率に影響した可能性がある．これらの点については今後の検討課題であり，さらなる症例の蓄積が必要であろう．NBNC-HCCには肝線維化や脂肪化に乏しく，NASHの関与だけでは説明できない成因不明な症例が存在する．現時点ではこれらCryptogenic-HCCの囲い込みは困難であるが，PIVKA IIや他臓器癌の合併が診断の手がかりになる可能性が示唆された．

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肝炎ウイルスによる発癌のメカニズム

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肝炎ウイルスによる発癌のメカニズム

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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]

●HCVはepigeneticな遺伝子変異(Rb, p53, WnT/β-カテニン, ATM, Chk2, 自然免疫系シグナル, 増殖因子シグナル)を介してC型肝発癌に関与している.

d. ATMとChk2

ataxia telangiectasia mutated(ATM) kinaseは二本鎖DNA断片を検出する癌抑制蛋白であり, DNA損傷チェックポイントを制御しているシグナル経路を調節している. HCV NS3/4Aプロテアーゼとの相互作用により, ATMは核内から細胞質の核近傍領域へ一部移行する. NS3/4Aは, 別のDNA損傷チェックポイントに関与する蛋白であるチェックポイント・キナーゼ(Chk2)と相互作用する可能性も報告されている. ATM, Chk2いずれのノックダウンによってもHCV-RNAの複製が阻害され, ウイルスは減少することから, これらの相互作用はウイルス複製を促進させるために引き起こされている可能性がある[19].

e. 自然免疫系シグナル

自然免疫系シグナルは腫瘍抑制機能と密接に関連していることがあるが, HCVは他のウイルス同様この自然免疫シグナル阻害作用をもつ. 例えばIFN誘導性のdsRNA-activated protein kinase(PKR)は転写因子であるeIF2αのリン酸化を阻害することでその翻訳を抑制し, 結果的に細胞増殖を抑制してアポトーシスを促進させる. すなわちPKRの抗腫瘍活性を示すものであるが, NS5A蛋白は*in vitro*ながらこのPKRに結合してその機能を抑制することが報告されている. またNS3/4Aプロテアーゼは Toll-like receptor 3やretinoic acid-inducible gene Iシグナル経路の両方に対するアダプター蛋白を標的とし, それにより細胞増殖抑制に関与する転写因子であるIFN調節因子3(IRF3)の活性化を阻害する. こうしたHCVによる自然免疫シグナル応答阻害はI型IFNを含む多数のIRF3依存性遺伝子の抗ウイルス作用からウイルスを守るために形成されたものであるが, 結果的にはHCVによる癌の進展にも影響を及ぼす可能性がある[19].

f. 増殖因子シグナル

増殖因子シグナル経路は, 肝細胞癌のイニシエーションや維持に重要な役割を持っている. transforming growth factor-β(TGF-β)シグナル経路は, 細胞増殖抑制もしくはpro-apoptticな影響を及ぼす. HCVコア蛋白はTGF-βにより活性化を受ける転写因子であるSmad3と相互作用をし, それによってTGF-βによる発癌抑制効果を阻害することが示唆されている[19].

おわりに●

肝発癌には多段階の過程が必要であり多くの細胞内シグナル伝達経路が関与している. HBVとHCV感染は肝細胞癌発生のための主要な危険因子であるが, ウイルス感染と肝細胞癌との間にどのような関連が存在するのかは未知なる部分が多い. これまでの研究からさまざまなウイルス蛋白が単独もしくは相互的に肝細胞癌進展に関与していることがわかってきた. 特にHCV蛋白は, 鉄代謝異常・脂質代謝異常・インスリン抵抗性といった代謝異常を引き起こし, ウイルス蛋白による直接的な酸化ストレスをさらに増強させ肝発癌をきたすことが知られている. またウイルス蛋白は, 細胞生存・増殖・分裂などにかかわる細胞内シグナル伝達にも関与している. 最近, マルチキナーゼ阻害薬であるソラフェニブは進行肝細胞癌患者において生存率を改善させることが報告されたが, ウイルス蛋白による細胞シグナル伝達の修飾という観点から肝細胞癌の発生機序を考えるうえでも興味深い結果と思われる. しかしながら, 肝発癌については肝炎ウイルスの直接的な機構についてもいまだcontroversialな部分も多く, 今後のさらなる分子機構の解明が必須と考えられる.

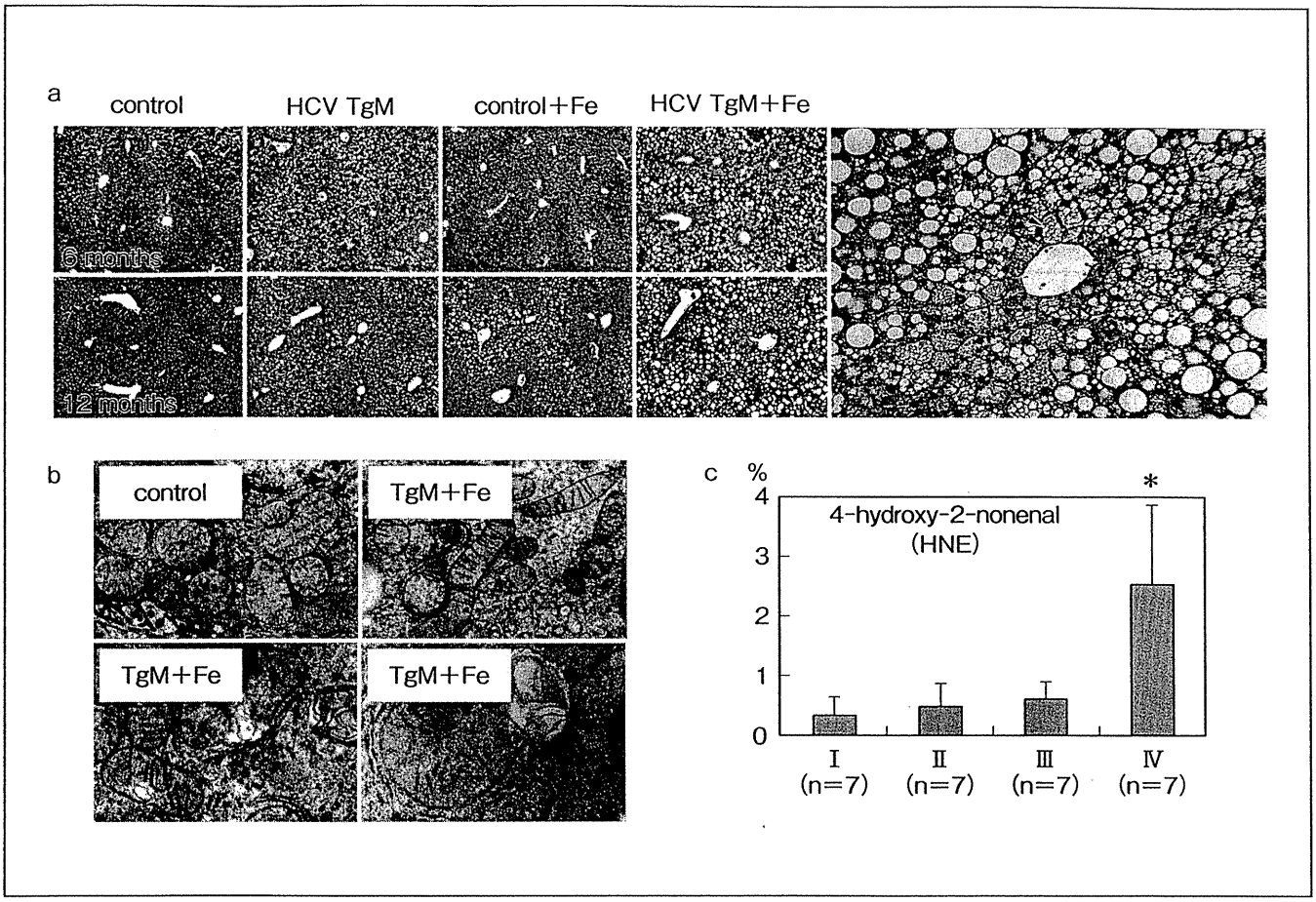

図1　鉄負荷されたHCVトランスジェニックマウス(HCV TgM)において，ミトコンドリア障害，肝脂肪化，を介して肝発癌をきたす　(次頁につづく)

a　通常餌のコントロールマウス(control)(Ⅰ群)，通常餌のTgM(HCV TgM)(Ⅱ群)，鉄負荷コントロールマウス(control＋Fe)(Ⅲ群)および鉄負荷HCV TgM(HCV TgM＋Fe)(Ⅳ群)の肝組織像(HE染色)．
中心静脈周囲には矢印で示すような小滴性脂肪の沈着が顕著である．

b　鉄負荷6ヵ月後のHCV TgM(TgM＋Fe)と通常餌のコントロールマウス(control)の肝ミトコンドア電子顕微鏡像．

c　鉄負荷12ヵ月目の肝組織内脂質過酸化物(4-hydroxy-2-nonenal：HNE)の比較．

d　12ヵ月目の肝組織内8-hydroxy-2′-deoxyguanosine(8-OHdG)量の比較．
鉄負荷HCV TgMの肝ミトコンドリアはコントロールに比べて大小不同や膜の膨化，クリステの乱れ，内膜腔のbuldgingなどの形態異常が認められる(b)．4-hydroxy-2-nonenal量は免疫染色による陽性面積の割合で比較した(c)．鉄負荷HCV TgMは他の3群に比べて8-OHdG量が多く，また他の3群もそれぞれ飼育開始時，6ヵ月目と比べて8-OHdG量は多かった(d)．

e　鉄負荷TgM(HCV TgM＋Fe)，コントロールマウス(control＋Fe)，通常餌TgM(HCV TgM)，コントロールマウス(control)の肝細胞癌発生率と鉄負荷TgM 12ヵ月目のマウス肝細胞癌写真．

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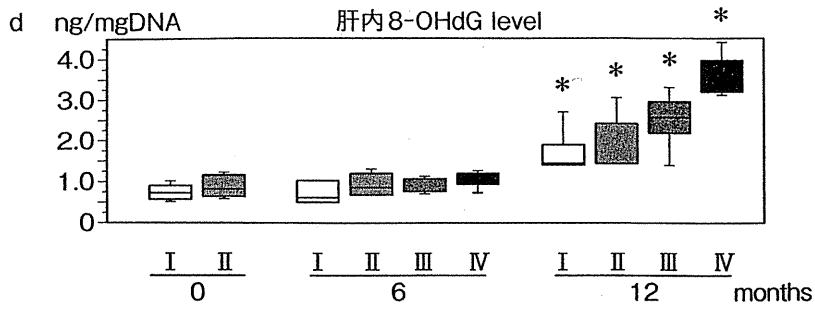
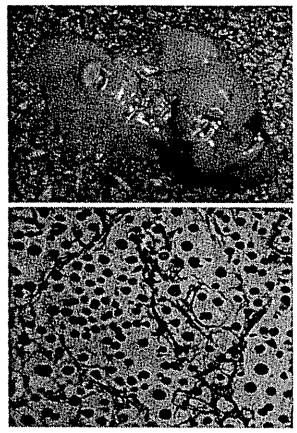


e



飼育期間	mice group	liver tumor
6 months	control	0/10
	control+Fe	0/10
	HCV TgM	0/10
	HCV TgM+Fe	0/10
9 months	control	0/7
	control+Fe	0/5
	HCV TgM	0/6
	HCV TgM+Fe	0/6
12 months	control	0/10
	control+Fe	0/9
	HCV TgM	0/8
	HCV TgM+Fe	5/11

図1 つづき

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