Hypervascular Hepatocellular Carcinoma: Correlation between Biologic Features and Signal Intensity on Gadoxetic Acid–enhanced MR Images

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Purpose: To analyze the correlation among biologic features, tumor marker production, and signal intensity at gadoxetic acid–enhanced MR imaging in hepatocellular carcinomas (HCCs).

Materials and Methods: Institutional ethics committee approval and informed consent were obtained for this retrospective study. From April 2008 to September 2011, 180 surgically resected HCCs in 180 patients (age, 65.0 years ± 10.3 [range, 34–83 years]; 138 men, 42 women) were classified as either hypointense (n = 158) or hyperintense (n = 22) compared with the signal intensity of the background liver on hepatobiliary phase gadoxetic acid–enhanced MR images. Pathologic features were analyzed and α fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II) production were compared by means of serum analysis and immunohistochemical staining. Recurrence and survival rates were also evaluated. The Mann-Whitney and Pearson correlation tests were used for statistical analysis.

Results: The grade of differentiation was higher (P = .028) and portal vein invasion was less frequent in hyperintense HCCs (13.6%) than in hypointense HCCs (36.7%) (P = .039). The serum levels of AFP, *Lens culinaris* agglutinin reactive fraction of AFP, and PIVKA-II were lower in hyperintense than in hypointense HCCs (P = .003, .004, and .026, respectively). Immunohistochemical AFP and PIVKA-II expression were lower in hyperintense than in hypointense HCCs (both $P < .001$). The recurrence rate was lower in hyperintense than in hypointense HCCs (P = .039).

Conclusion: The results suggest that hyperintense HCCs on gadoxetic acid–enhanced MR images are less aggressive than hypointense HCCs.

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Hepatocellular carcinoma (HCC) is the most frequent primary malignant hepatic tumor and the third most common cause of cancer death worldwide (1). The accurate detection and characterization of HCC are critical issues in clinical practice for improving the prognosis of patients with HCC.

Gadoxetic acid–enhanced MR imaging is a new imaging modality with high accuracy for diagnosing HCCs (2–4). On images obtained during the hepatobiliary phase of gadoxetic acid–enhanced MR imaging, HCCs commonly show hypointensity when compared with the background liver. However, approximately 6%–15% of hypervascular HCCs demonstrate iso- or hyperintensity, which is uncommon among hepatic malignant tumors (5–8). This hyperintensity was previously shown to be due to overexpression of organic anion transporting polypeptide 8 (OATP8, synonymous with OATP1B3), which might be the uptake transporter of gadoxetic acid in HCCs (5,6). In the normal liver, OATP8 is expressed on the sinusoidal side of the hepatocyte membrane and takes up many intrinsic and extrinsic organic anions from blood into hepatocytes.

On the other hand, Jung et al (9) showed that OATP8 was up-regulated by hepatocyte nuclear factor 1α. These hepatocyte nuclear factors are indispensable transcription factors that relate to primitive embryonal differentiation of hepatocytes and to hepatocarcinogenesis. We suspected that these atypical hypervascular HCCs that show hyperintensity on hepatobiliary phase images (hyperintense HCC) might reflect a distinct subtype of HCC with a particular molecular background and biologic features.

The main tumor marker of HCCs is α-fetoprotein (AFP), especially the *Lens culinaris* agglutinin reactive fraction (L-3). Similarly, the protein induced by vitamin K absence or antagonist-II (PIVKA-II) is a clinically important serum tumor marker. PIVKA-II is an incomplete coagulation factor prothrombin II whose production is related to the absence of vitamin K or the presence of the antagonist of vitamin K, which is the cofactor of γ carboxylase that converts precursor into prothrombin (10). Serum levels of both AFP and PIVKA-II correlate with the histologic degree of malignancy and the prognosis in HCC (11). In addition, there are reports (12,13) showing that AFP expression in HCCs is regulated by several enhancers and suppressors, including the hepatocyte nuclear factor family. Although the molecular basis of PIVKA-II production is not well explained, we speculated that there might be a correlation of the tumor marker production and signal intensity (SI) on hepatobiliary phase images, which would reflect distinct genomic and proteomic expression of HCC.

The purpose of this study was to analyze the correlation among the pathologic and biologic features, tumor marker production, with SI on hepatobiliary phase gadoxetic acid–enhanced MR images of HCCs.

Advances in Knowledge

- Hypervascular hepatocellular carcinomas (HCCs) that hyperintensity relative to the surrounding liver on hepatobiliary phase gadoxetic acid–enhanced MR images demonstrate a significantly higher grade of differentiation ($P = .028$) and rarer portal vein invasion ($P = .039$) than those of hypointense HCCs.
- Hyperintense HCCs on hepatobiliary phase gadoxetic acid–enhanced MR images show significantly lower serum level of α fetoprotein, *Lens culinaris* agglutinin reactive fraction of α fetoprotein, and protein induced by Vitamin K absence or antagonist-II than hypointense HCCs ($P = .003$, $P = .004$, and $P = .026$, respectively).
- Hyperintense HCCs on hepatobiliary phase gadoxetic acid–enhanced MR images similarly show significantly weaker expression of α fetoprotein and protein induced by Vitamin K absence or antagonist-II at immunohistochemical evaluation than did hypointense HCCs (both $P < .001$).
- Hyperintense HCCs on gadoxetic acid–enhanced MR images show a significantly lower recurrence rate than do hypointense HCCs ($P = .039$).

Implication for Patient Care

- Hypervascular HCCs that show hyperintensity on hepatobiliary phase gadoxetic acid–enhanced MR images have biologically less aggressive features than do those that show hypointensity.

Materials and Methods

Patients

This retrospective study received the approval of the institutional ethics committee, and informed consent for using the MR images and resected specimens was obtained from all patients. There were 207 consecutive patients who had 233 HCCs that were surgically resected

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Abbreviations:
AFP = α-fetoprotein
HCC = hepatocellular carcinoma
L-3 = *Lens culinaris* agglutinin reactive fraction
mAU = milli–arbitrary unit
OATP = organic anion transporting polypeptide
PIVKA-II = protein induced by vitamin K absence or antagonist-II
SI = signal intensity
TR = repetition time

Author contributions:
Guarantors of integrity of entire study, A.K., O.M., K.K., S. Kobayashi, T.G., S. Kaneko, Y.N., S.A.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; literature research, A.K., O.M., N.Y., K.K., S. Kobayashi, S. Kaneko, Y.N., S.A.; clinical studies, A.K., O.M., K.K., S. Kobayashi, W.K., T.G., T.Y., S. Kaneko, R.K., S.A.; experimental studies, A.K., N.Y., Y.N., S.A.; statistical analysis, A.K.; and manuscript editing, A.K., O.M., S. Kobayashi, T.G., S. Kaneko, S.A.

Conflicts of interest are listed at the end of this article.

at our institution and six affiliated institutions from April 2008 to September 2011. Patients were excluded if they had more than one HCC (12 patients with 31 nodules), if they had previous treatment (three patients with 10 nodules), if they did not have MR imaging (nine patients with nine nodules) or if their lesions were hypovascular in the arterial phase (three patients with three nodules) (Fig E1 [online]). Average age was 65.0 years ± 10.3 (range, 34–83) (age of men 64.5 years ± 10.5 [range, 34–83 years]; women, 67.4 years ± 9.5 [43–83 years]). Ratio of men to women was 138 (76.7%) to 42 (23.3%). The background liver was normal in 28 patients, whereas 70 patients had chronic hepatitis and 82 had cirrhosis. The origin of liver disease was viral hepatitis type B in 41 patients, type C in 85, types B and C in two, alcoholism in nine, and other origins in 43. Hepatic function was classified as Child-Pugh class A in 169 patients and class B in 11. Average tumor size was 33.8 mm ± 23.4 (range, 7–160 mm).

Gadoxetic Acid–enhanced MR Imaging

Gadoxetic acid–enhanced MR imaging was performed 52.8 days ± 25.3 [range, 3–95 days] before surgical resection for the characterization and pretreatment staging of HCC. MR images were obtained on several MR systems: Signa HDx 1.5 T and 3 T (GE Medical Systems, Milwaukee, Wis), Intera Achieva 1.5 T (Philips Medical Systems, Best, Netherlands), Symphony 1.5 T (Siemens, Erlangen, Germany) and Magnetom Vision 1.5 T (Siemens). MR imaging was performed with fat-suppressed two-dimensional or three-dimensional gradient-echo T1-weighted sequences (repetition time, 3.2–4.0 msec; echo time, 1.6–2.3 msec; flip angle, 10–15 degrees; field of view, 33–42 cm; matrix, 128–192 interpolated to 256–512; section thickness, 4.0–8.0 mm). For dynamic study, a dose of 0.1 mL per kilogram of 0.25 mmol/mL of gadoxetic acid (Primovist, Bayer Schering Pharma, Berlin) was injected intravenously at a flow rate of 1–2.0 mL per second, followed by a 20–40 mL saline flush. To obtain the optimal arterial dominant phase, the following methods were used. In the bolus tracking method, arterial phase timing was determined as the peak time of the abdominal aorta plus 7–15 seconds. In the test injection method (1.5 mL of Primovist + 8 mL saline flush), arterial-phase timing was determined as the peak time of the abdominal aorta plus 10 seconds minus half of imaging time. Portal phase and equilibrium phase images were obtained at 60–90 seconds and 120–180 seconds after injection, respectively. The hepatobiliary phase images were obtained 15–20 minutes after the injection.

Analysis of SI on Gadoxetic Acid–enhanced MR Images

Image analysis was performed by two abdominal imaging radiologists (A.K. and O.M., with 10 and 40 years of experience, respectively) without information on clinical and pathologic results. The SI of the tumor and surrounding background liver was individually measured and then averaged by placing regions of interest during the hepatobiliary phase. The region of interest of the tumor was determined as the maximum oval or round area at the level of the largest diameter of the tumor, avoiding degeneration area and artifact. The average size of the region of interest was 923.6 mm^2 ± 1418.3 (range, 61–6167 mm^2). The average intensity of the entire region of interest was used for analysis. A region of interest of the same size as the tumors was placed on the adjacent liver parenchyma, avoiding the large vessels.

Hypointense HCC was defined as showing lower SI than that of the surrounding liver (tumor SI/background SI < 1.0) (Fig 1a), and hyperintense HCC as showing equal or higher SI (tumor SI/background SI ≥ 1.0) (Fig 2a).

The Enhancement Ratio in the Hepatobiliary Phase

To evaluate the uptake level of gadoxetic acid, we calculated the enhancement ratio of HCCs in the hepatobiliary phase. We could not consistently assess all patients because they were examined with various MR systems and by using somewhat different parameters. As a result, we focused on only 79 HCCs studied at our institution because they were imaged by a variable flip angle method for measuring T1 value. MR images were obtained with either a 1.5-T or 3-T MR system (Signa HDx; GE Medical Systems, Milwaukee, Wis). MR imaging was performed with fat-suppressed three-dimentional spoiled gradient-echo in the steady state T1-weighted sequences (liver acquisition with volume acceleration; generalized encoding matrix; repetition time, 3.2–4.0 msec; echo time, 1.6 msec; flip angle, 6–15 degrees; field of view, 42 × 42 cm; matrix, 192 × 320, interpolated to 512 × 512; section thickness, 4.2 mm; overlap, 2.1 mm). The unenhanced phase was imaged with two different flip angles to calculate the static T1 value. The hepatobiliary phase images were obtained 20 minutes after the injection. The static T1 value before enhancement ($T1_{pre}$) was calculated as follows:

$$\exp(-TR/T1_{pre}) = (SI_A \sin\beta - S_B \sin\alpha) / (SI_A \sin\beta \cos\alpha - SI_B \sin\alpha \cos\beta)$$

where TR is repetition time and SI_A and SI_B represent the signal intensity in flip angle α and β, respectively. The enhanced images were obtained with the same parameters by using flip angle α. Then the T1 value after enhancement ($T1_{post}$) was calculated as follows:

$$\exp(-TR/T1_{post}) = \{SI_{pre}[1-\exp(-TR/T1_{pre})\cos\alpha] + SI_{post}[\exp(-TR/T1_{pre})-1]\} / \{SI_{pre}[1-\exp(-TR/T1_{pre})\cos\alpha] + SI_{post}[\exp(-TR/T1_{pre})-1]\cos\alpha\}$$

The enhancement ratio was shown as (8)

$$(1/T1_{post} - 1/T1_{pre}) / (1/T1_{pre})$$

Histologic Diagnosis

Hematoxylin and eosin staining was carried out in tissue sections of all 180

Figure 1

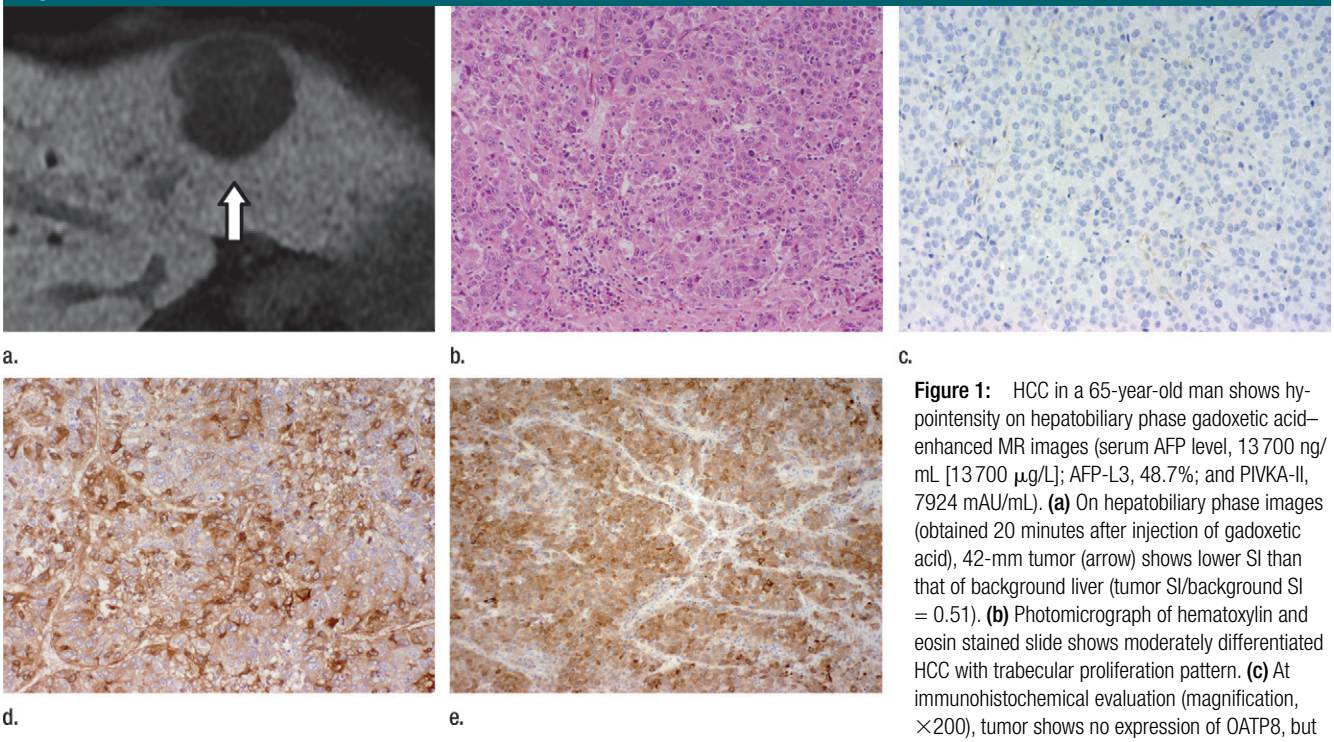

Figure 1: HCC in a 65-year-old man shows hypointensity on hepatobiliary phase gadoxetic acid–enhanced MR images (serum AFP level, 13 700 ng/mL [13 700 μg/L]; AFP-L3, 48.7%; and PIVKA-II, 7924 mAU/mL). **(a)** On hepatobiliary phase images (obtained 20 minutes after injection of gadoxetic acid), 42-mm tumor (arrow) shows lower SI than that of background liver (tumor SI/background SI = 0.51). **(b)** Photomicrograph of hematoxylin and eosin stained slide shows moderately differentiated HCC with trabecular proliferation pattern. **(c)** At immunohistochemical evaluation (magnification, ×200), tumor shows no expression of OATP8, but **(d)** intense expression of both AFP (brown color) and **(e)** PIVKA-II (brown color).

liver specimens. HCCs were diagnosed by consensus of two liver pathologists (S.K and Y.N. with 10 and 38 years of experience, respectively), according to the classification proposed by the International Working Party (14) and the World Health Organization classification (15). We compared hypointense HCCs and hyperintense HCCs with regard to histologic features such as macroscopic growth patterns (indistinct margin, simple nodular, extranodular growth, and multinodular patterns) (16), differentiation grade (well, moderately, and poorly differentiated), proliferation pattern (trabecular, pseudoglandular, scirrhous, and compact pattern), fibrous capsule invasion, portal vein invasion and hepatic vein invasion.

Measuring Serum Levels of AFP and PIVKA-II

Preoperative patient serum levels were obtained 10.2 days ± 7.3 (range, 0–35 days) before or after MR imaging. Serum AFP levels were measured by using chemiluminescent enzyme immunoassay (Lumipulse presto; Fujirebio, Tokyo, Japan). Serum AFP-L3 levels were measured by means of liquid-phase binding assay-electrokinetic analyte transport assay (LBA AFP-L3; Wako Pure Chemical Industries, Osaka, Japan), and were expressed as the ratio of AFP-L3 to total AFP percentage. Serum PIVKA-II levels were measured by electrochemiluminescence immunoassay (Picolumi PIVKA-II; Eidia, Tokyo, Japan) and were expressed in milli–arbitrary units (mAUs).

Immunohistochemical Analysis of AFP, PIVKA-II, and OATP8

Immunostaining was performed for all HCC specimens by using the primary antibodies human AFP (rabbit polyclonal; DAKO, Glostrup, Denmark), human PIVKA-II (MU-3 mouse monoclonal; Eidia, Tokyo, Japan) and human OATP8 (mouse monoclonal NB100-74482; Novus Biologicals, Littleton, Colo).

Two abdominal imaging radiologists (N.Y. and A.K., with 9 and 10 years of experience, respectively, in radiology and pathologic research) independently and blindly evaluated the intensity of the AFP and PIVKA-II expression on tumor cytoplasm as follows: grade 0, no expression; grade 1, weak expression; grade 2, moderate expression; and grade 3, strong expression. Similarly, they semiquantitatively evaluated the intensity of OATP8 expression on tumor cellular membranes compared with the background hepatocytes as follows: grade 0, no expression; grade 1, decreased expression; grade 2, equivalent expression; and grade 3, increased expression. We analyzed the average grades of the two investigators.

Then, we compared hypointense and hyperintense HCCs for clinical and histologic features and AFP/PIVKA-II expression (serum level and immunohistochemical analysis). To examine whether the AFP and PIVKA level simply correlates with the differentiation grade of HCCs, we performed the same analysis excluding 42 poorly differentiated HCCs. We analyzed the correlation

Figure 2

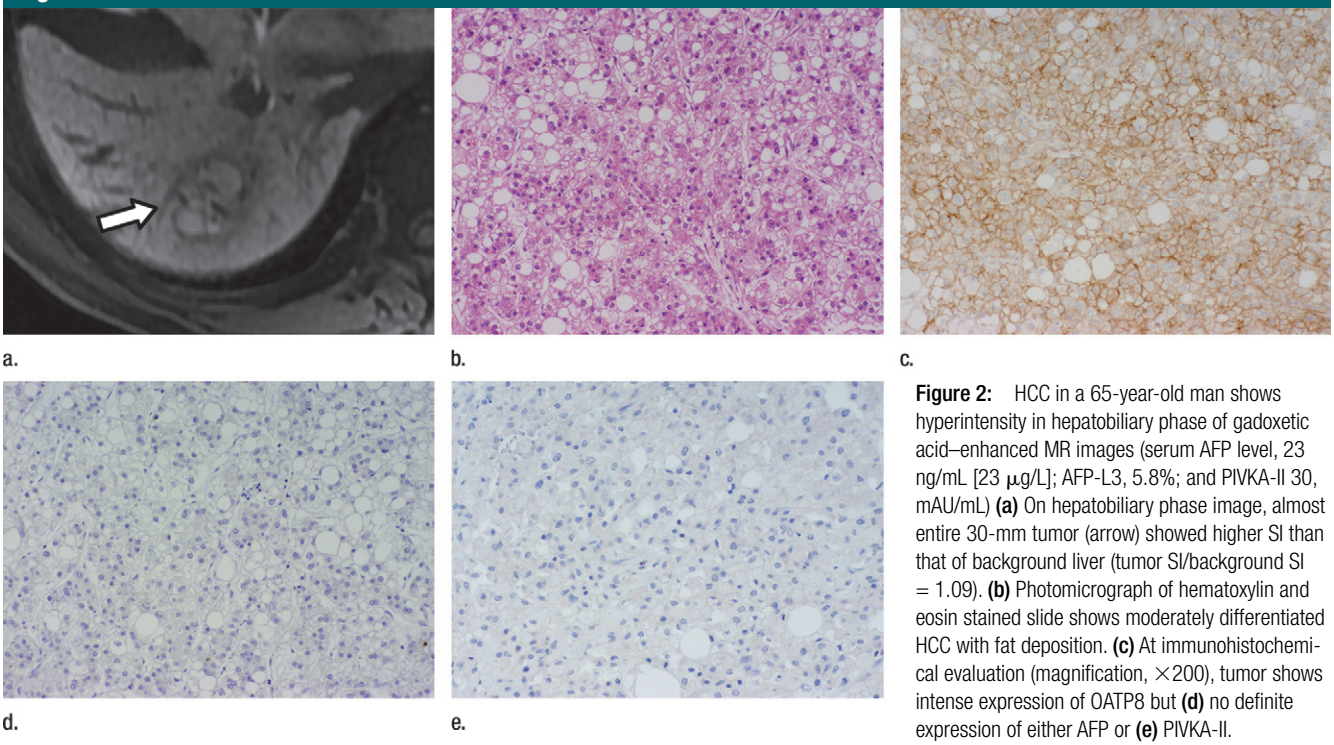

Figure 2: HCC in a 65-year-old man shows hyperintensity in hepatobiliary phase of gadoxetic acid–enhanced MR images (serum AFP level, 23 ng/mL [23 μg/L]; AFP-L3, 5.8%; and PIVKA-II 30, mAU/mL) **(a)** On hepatobiliary phase image, almost entire 30-mm tumor (arrow) showed higher SI than that of background liver (tumor SI/background SI = 1.09). **(b)** Photomicrograph of hematoxylin and eosin stained slide shows moderately differentiated HCC with fat deposition. **(c)** At immunohistochemical evaluation (magnification, ×200), tumor shows intense expression of OATP8 but **(d)** no definite expression of either AFP or **(e)** PIVKA-II.

among the immunohistochemical AFP, PIVKA-II, and OATP8 expression. We also analyzed the correlation among enhancement ratio and serum levels and immunohistochemical AFP and PIVKA-II expression for 79 HCCs.

Recurrence and Survival Rates in Patients with HCC

We compared the two groups for recurrence (including all local recurrence and intrahepatic and extrahepatic metastasis) and survival duration from the operation day. The follow-up length was 727 days ± 365 (range, 22–1293 days). When intrahepatic hypervascular HCCs or obvious extrahepatic metastasis appeared on follow-up dynamic computed tomography or gadoxetic acid–enhanced MR imaging, we considered it to be recurrence.

Statistical Analyses

Statistical significance was evaluated with GraphPad Prism5 (GraphPad Software, San Diego, Calif) and Excel Statistics 2010 (Social Survey Research Information, Tokyo, Japan). We used the Fisher test for the analysis of the clinical and histologic features; Mann-Whitney test for the comparison of serum and immunohistochemical tumor marker levels; Pearson correlation test for the correlations among AFP, PIVKA-II, OATP8 expression and enhancement ratio; and κ test for the evaluation of interobserver variation in the analysis of immunohistochemistry. The κ test score (the level of agreement) was defined as follows: 0.0–0.40, poor; 0.41–0.60, moderate; 0.61–0.80, good to fair; and 0.81–1.0, excellent. Kaplan-Meier analysis with Log-rank test, logistic regression, and Cox regression were performed for the evaluation of clinical outcome and recurrence. A *P* value less than 0.05 was considered to indicate a statistically significant difference.

Results

Clinical Features of the Two Types of HCC

One hundred and fifty-eight nodules were classified as hypointense HCCs (average tumor SI/background SI, 0.46 ± 0.11; range, 0.24–0.67) and the remaining 22 nodules were classified as hyperintense HCC (average tumor SI/background SI, 1.19 ± 0.22; range, 1.06–1.86). No significant differences were observed in clinical features such as sex, background liver, liver function, or tumor size between the patients with hypointense HCC and hyperintense HCC, but there was a significant difference for age (Table 1). The patients with hyperintense HCCs were significantly older than those with hypointense HCCs ($P = .04$).

Pathologic Features of the Two Types of HCC

None of the differences noted in the macroscopic growth patterns between the hypointense and hyperintense HCCs were significant ($P = .77$) (Fig E2a [online]). The hyperintense HCCs showed significantly higher differentiation grade than the hypointense HCCs ($P = .028$) (Fig E2b [online]). Pseudoglandular pattern was more frequently seen in hyperintense HCCs than in hypointense HCCs (Fig E2c [online]). There was a significant difference in the proliferation

Table 1

Clinical Features of Patients

Clinical Features	Hypointense HCCs	Hyperintense HCCs	*P* Value
No. of tumors	158	22	
Resected tumor size (mm)	33.2 ± 22.9 (7–160)*	37.7 ± 18.9 (10–105)*	.38
Age (y)	64.6 ± 10.3 (34–83)*	69.5 ± 7.8 (52–81)*	.04
Sex			.30
Men	119	3	
Women	39	19	
Background liver tissue			.23
Normal liver	23	5	
Chronic hepatitis	65	5	
Liver cirrhosis	70	12	
Origin of liver disease			.10
Hepatitis B	38	3	
Hepatitis C	74	11	
Hepatitis B and C	2	0	
Alcoholism	6	3	
Other	38	5	
Child Pugh classification			.63
A	149	20	
B	9	2	

Note.—Unless otherwise indicated, data are number of patients.

* Data are means ± standard deviations, with ranges in parentheses.

pattern between the hypointense and hyperintense HCCs ($P < .001$). The hypointense HCCs showed higher positive rates for fibrous capsule invasion and hepatic vein invasion, although the differences did not reach statistical significance ($P = .81$ and .21, respectively). The hyperintense HCCs showed a significantly lower rate of portal vein invasion than that of hypointense HCCs ($P = .039$) (Fig E2e [online]).

Serum Levels of AFP, AFP-L3 Fraction, and PIVKA-II

The serum levels of tumor markers AFP, AFP-L3, and PIVKA-II were significantly lower in the patients with hyperintense HCCs than in those with hypointense HCCs ($P = .003$, .004, and .026) (Figs 3, E3 [online]). To examine whether the AFP and PIVKA levels correlated with the differentiation grade of the HCCs, we performed the same analysis, excluding 42 poorly differentiated HCCs (Fig E4 [online]). Despite excluding poorly differentiated HCCs, the serum levels of these markers were also lower in the patients with hyperintense HCCs than in those with hypointense HCCs ($P = .005$, .019, and .08).

Immunohistochemistry of AFP and PIVKA-II in HCCs

In the semiquantitative analyses of immunohistochemical OATP8, AFP, and PIVKA-II, interobserver agreement of the two readers was good to excellent ($\kappa = 0.82$, 0.78, and 0.81, respectively). In immunohistochemical analysis, OATP8 expression was significantly decreased in hypointense HCCs compared with that in hyperintense HCCs ($P < .001$) (Fig 4a). The AFP expression was significantly higher in hypointense HCCs than that in hyperintense HCCs ($P < .001$) (Fig 4b). There was a significant negative correlation between AFP expression and OATP8 expression ($P = .002$, $R = -0.22$) (Fig E5c [online]). The immunohistochemical PIVKA-II expression was also significantly higher in hypointense HCCs than that in hyperintense HCCs ($P < .001$) (Fig 4c). There was a significant negative correlation between PIVKA-II expression and OATP8 expression ($P < .001$, $R = -0.38$) (Fig E5e [online]). We also performed the same immunohistochemical analysis excluding poorly differentiated HCCs (Fig E6 [online]). The expression of OATP8 was significantly lower, but expression of AFP and PIVKA-II was significantly higher in hypointense HCCs than those in hyperintense HCCs (both $P < .001$). There was still a significant negative correlation between AFP and OATP8 expression ($P = .0017$, $R = -0.27$) and between PIVKA-II and OATP8 expression ($P < .001$, $R = -0.46$).

Relative Enhancement Ratio on Hepatobiliary Phase and AFP or PIVKA-II Production

We analyzed the correlation between relative enhancement ratio and tumor marker expression for 79 HCCs (69 hypointense and 10 hyperintense HCCs). Significant negative correlations were noted among the enhancement ratio and serum AFP ($P = .023$, $R = -0.25$), serum AFP-L3 ($P < .001$, $R = -0.49$) and serum PIVKA-II level ($P = .018$, $R = -0.26$) (Fig E7a, E7b [online]). At immunohistochemical analysis, we also confirmed significant negative correlations among the enhancement ratio and AFP expression ($P = .007$, $R = -0.30$) and PIVKA-II expression ($P = .009$, $R = -0.29$) (Fig E7d, E7e [online]).

Analysis of Prognosis in Patients with HCC

The patients with hyperintense HCCs showed a significantly lower recurrence rate than those with hypointense HCCs ($P = .039$). The patients with hyperintense HCCs tended to show longer survival than those with hypointense HCCs, although without significant difference ($P = .07$) (Fig 5). Clinical features such as age and tumor size did not affect the recurrence and survival curves (Table E1 [online]). The summary of results is shown in Table 2.

Discussion

In our study, hyperintense HCCs in the hepatobiliary phase showed sig

Figure 3

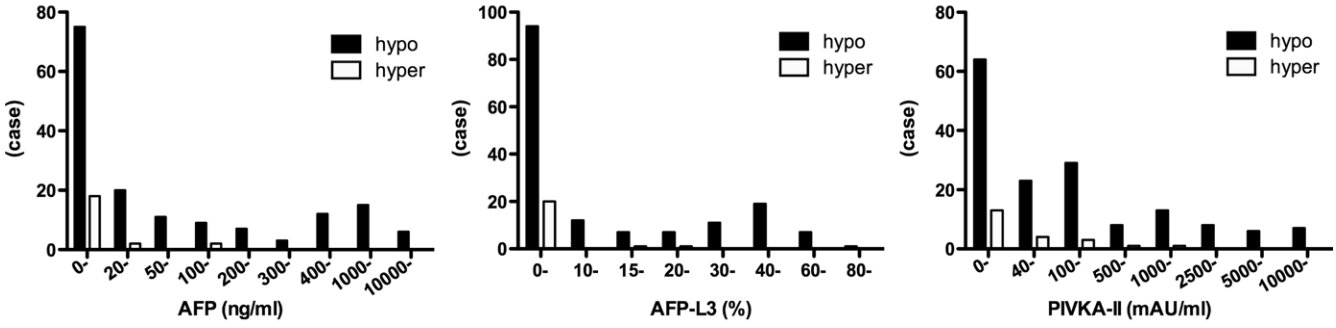

Figure 3: Graphs show serum levels of AFP, AFP-L3, and PIVKA-II. **(a)** Serum level of AFP was in normal range (<20 ng/mL [<20 μg/L]) in 18 of 22 patients (82%) with hyperintense HCCs and in 75 of 158 (47%) of patients with hypointense HCCs. Average serum AFP value was 1202.7 ng/mL ± 4369.9 [1202.7 μg/L ± 4369.9] in patients with hypointense HCCs and 17.9 ng/mL ± 29.0 [17.9 μg/L ± 29.0] in patients with hyperintense HCC, ($P = .003$). **(b)** Serum level of AFP-L3 was in normal range (<10%) in 20 of 22 patients (91%) with hyperintense HCCs, and 94 of 158 (59%) patients with hypointense HCCs. Average serum AFP-L3 fraction value was significantly lower in patients with hyperintense HCC (3.8% ± 7.5) than in those with hypointense HCC (15.9% ± 21.2) ($P = .004$). **(c)** Serum level of PIVKA-II was in normal range (<40 mAU/mL) in 13 of 22 (59%) patients with hyperintense HCCs, and 64 of 158 (40%) patients with hypointense HCCs. The serum level of PIVKA-II was also lower in patients with hyperintense HCCs (190.6 mAU/mL ± 468.6) than those with hypointense HCCs (1697.9 mAU/mL ± 6232.0) ($P = .026$).

Figure 4

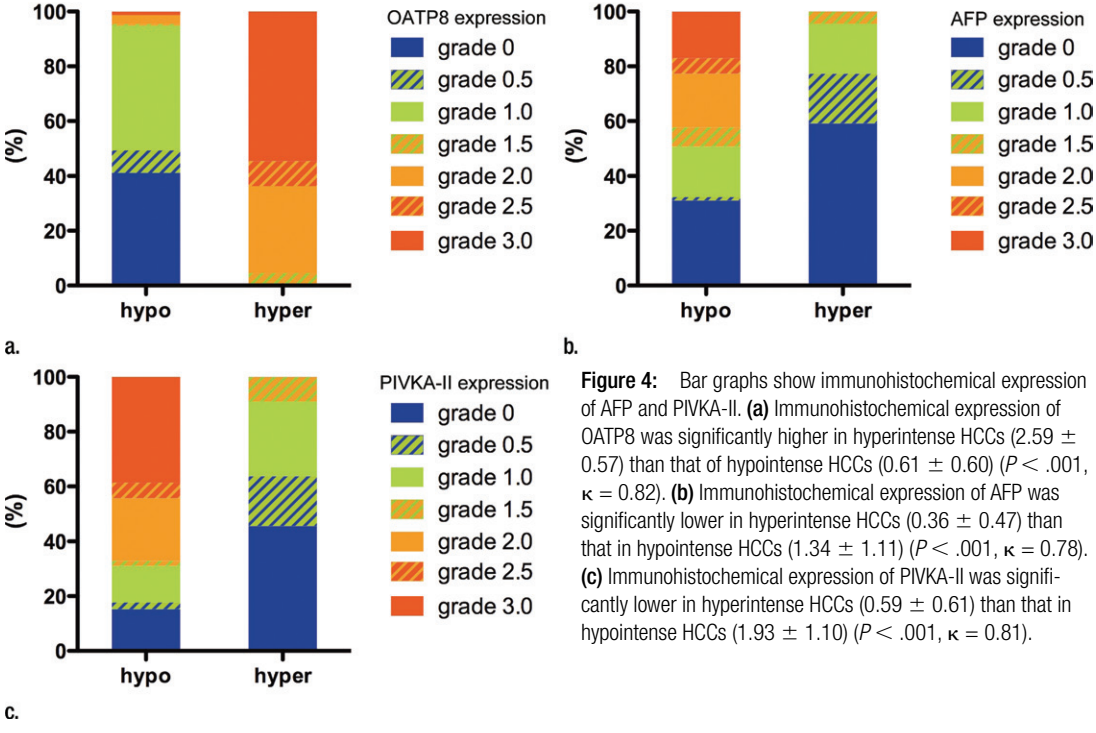

Figure 4: Bar graphs show immunohistochemical expression of AFP and PIVKA-II. **(a)** Immunohistochemical expression of OATP8 was significantly higher in hyperintense HCCs (2.59 ± 0.57) than that of hypointense HCCs (0.61 ± 0.60) ($P < .001$, $\kappa = 0.82$). **(b)** Immunohistochemical expression of AFP was significantly lower in hyperintense HCCs (0.36 ± 0.47) than that in hypointense HCCs (1.34 ± 1.11) ($P < .001$, $\kappa = 0.78$). **(c)** Immunohistochemical expression of PIVKA-II was significantly lower in hyperintense HCCs (0.59 ± 0.61) than that in hypointense HCCs (1.93 ± 1.10) ($P < .001$, $\kappa = 0.81$).

nificantly higher differentiation grades with lower frequency of portal vein invasion than did the hypointense HCCs. Moreover, hyperintense HCCs showed significantly lower expression of AFP and PIVKA-II than did hypointense HCCs. AFP and PIVKA-II levels correlated with the histologic grade of malignancy and poor prognosis (11); however, we demonstrated that the difference in tumor marker production between hypointense HCCs and hyperintense HCCs did not depend on the differentiation grade when poorly differentiated HCCs were excluded

Figure 5

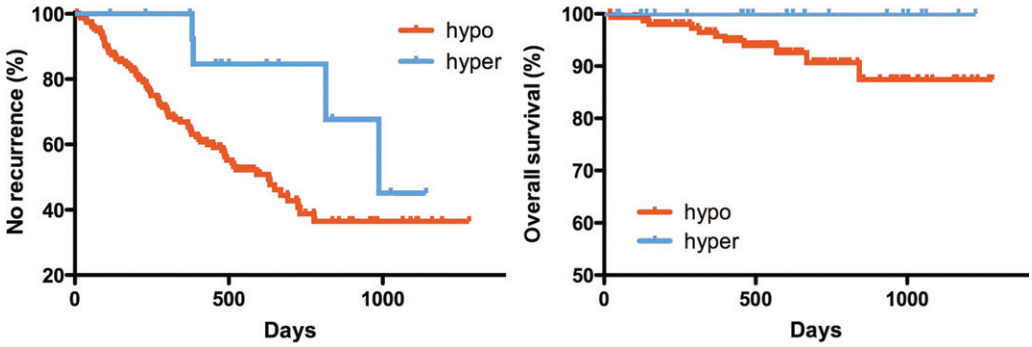

Figure 5: Charts show prognosis of patients with HCC. **(a)** Patients with hyperintense HCCs showed significantly lower recurrence rate (6 of 22, 27.2%) than did those with hypointense HCCs (83 of 158, 52.5%) ($P = .039$). **(b)** Patients with hyperintense HCCs tended to show longer survival (mortality, 0 of 22, 0%) than those with hypointense HCCs (22 of 158, 13.9%). However, there was no significant difference between the two groups ($P = .07$).

Table 2

Summary of Results

Result	Hypointense HCCs ($n = 158$)	Hyperintense HCCs ($n = 22$)	*P* Value
Macro growth pattern			.77
Indistinct margin	6	0	
Simple nodular	102	17	
Extranodular	30	3	
Multinodular	20	2	
Differentiation			.028
Well differentiated	22	4	
Moderately differentiated	94	18	
Poorly differentiated	42	0	
Proliferation pattern			<.001
Trabecular	116	12	
Pseudoglandular	20	10	
Schirrous	9	0	
Compact	13	0	
Fibrous capsule invasion	63 (39.9%)	8 (36.4%)	.810
Portal vein invasion	58 (36.7%)	3 (13.6%)	.039
Hepatic vein invasion	21 (13.3%)	0 (0%)	.210
Serum levels			
AFP (ng/mL)	1202.7 ± 4369.9 (497.4 ± 1899.1*)	17.9 ± 29.0	.003 (.005*)
AFP-L3 (%)	15.9 ± 21.2 (14.5 ± 21.0*)	3.8 ± 7.5	.004 (.019*)
PIVKA-II (mAU/mL)	1697.9 ± 6232.0 (1497.6 ± 7067.9*)	190.6 ± 468.6	.026 (.08*)
Immunohistochemical analysis			
OATP8	0.61 ± 0.60 (0.67 ± 0.63*)	2.59 ± 0.57	<.001 (<.001*)
AFP	1.34 ± 1.11 (1.34 ± 1.10*)	0.36 ± 0.47	<.001 (<.001*)
PIVKA-II	1.93 ± 1.10 (1.87 ± 1.08*)	0.59 ± 0.61	<.001 (<.001*)
Recurrence rate	83 (52.5%)	6 (27.2%)	.039
Survival rate	22 (86.1%)	22 (100%)	.070

* Excluding poorly differentiated HCCs ($n = 42$).

from the analysis. We suspect that the molecular regulatory mechanism of OATP8 expression may have some common channels with those of AFP or PIVKA-II expression.

In addition, hyperintense HCCs on hepatobiliary phase images showed a significantly lower recurrence rate than did hypointense HCCs. The patients with hyperintense HCCs showed longer survival than those with hypointense HCCs, but the difference was not statistically significant. In our study, the follow-up period averaged 727 days, which might not have been sufficient to demonstrate a significant difference.

Several prior reports have suggested that transcription factor hepatocyte nuclear factors control both OATP8 and AFP expression (9,12,13,17). Therefore, we speculated that some correlation between OATP8 and AFP expression through the hepatocyte nuclear factor family might exist. The regulatory mechanism of PIVKA-II and the correlation with OATP8 expression in HCC have not yet been determined. The transcription of the OATP8 gene is also regulated by the nuclear factor (steroid and xenobiotics receptor or pregnane xenobiotics receptor) (18). Vitamin K can be the ligand of these receptors, and it regulates the transcription of target genes (19). If vitamin

K decreases in HCC, the PIVKA-II production increases (20). We suspected that the transcription of OATP8 might change in accordance with the decrease of ligands to these receptors. As a result, we expected that PIVKA-II expression and OATP8 expression might be correlated.

However, there were many patients that showed low AFP and PIVKA-II expression in hypointense HCCs. We attribute this to the fact that OATP8, AFP, and PIVKA-II have several direct and indirect regulatory mechanisms other than the hepatocyte nuclear factor route (21). Further investigation is needed to clarify the real underlying molecular biology of these correlations.

The molecular classification of subtypes of HCCs is now being investigated by several groups (22). Yamashita et al (23,24) reported on HCC subtypes that were classified on the basis of expression of AFP and epithelial cell adhesion molecule, a stem cell marker. According to their reports, AFP-positive and epithelial cell adhesion molecule–positive HCCs showed stem and progenitor cell features with invasive character and poor prognosis compared with AFP-negative and epithelial cell adhesion molecule–negative HCCs that demonstrated mature hepatocyte-like features with a relatively good prognosis. These features of AFP-negative and epithelial cell adhesion molecule–negative HCCs resembled those of hyperintense HCCs, and we surmised that the origin of hyperintense HCC may be mature hepatocyte-like cells rather than stem or progenitor cells. We think that hyperintense HCCs may have some specific molecular or genetic profiles. Further molecular and genetic analyses are needed to clarify the exact molecular biologic basis for this possible subtype of HCCs.

Our study had limitations. First, the total number of hyperintense HCCs examined was small because such tumors are relatively rare (8). Second, we only assessed HCC lesions that were hypervascular in the arterial phase, and therefore our results cannot be applied to a general screening population with benign disease, hypovascular HCC on arterial-phase images, or other malignancies. Third, there was a variability of the imaging parameters, such as strength of magnetic field, section thickness, and on imaging timing, because this was multicenter study.

In conclusion, hyperintense HCCs on hepatobiliary phase images showed significantly higher differentiation grades, less frequent portal vein invasion, and lower recurrence rates than did hypointense HCCs. Moreover, hyperintense HCCs showed significantly lower expression of AFP and PIVKA-II than did hypointense HCCs. Hyperintense HCCs on hepatobiliary phase gadoxetic acid–enhanced MR images may be a particular form of hypervascular HCC with biologically less aggressive features than those of hypointense HCCs.

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