Dysplastic Nodules Frequently Develop into Hepatocellular Carcinoma in Patients with Chronic Viral Hepatitis and Cirrhosis

Masahiro Kobayashi, M.D.
Kenji Ikeda, M.D.
Tetsuya Hosaka, M.D.
Hitomi Sezaki, M.D.
Takashi Someya, M.D.
Norio Akuta, M.D.
Fumitaka Suzuki, M.D.
Yoshiyuki Suzuki, M.D.
Satoshi Saitoh, M.D.
Yasui Arase, M.D.
Hiromitsu Kumada, M.D.

Department of Gastroenterology, Toranomon Hospital, Tokyo, Japan.

Supported in part by a Research Grant from the Japanese Ministry of Health, Labor and Welfare, and Okinaka Memorial Foundation of Toranomon Hospital.

Address for reprints: Masahiro Kobayashi, M.D., Department of Gastroenterology, Toranomon Hospital, 2–2-two Toranomon, Minato-ku, Tokyo 105–8470, Japan; Fax: (011) 81 (44) 860–1623. E-mail: mshkobayashi@toranomon.gr.jp

Received February 25, 2005; revision received June 21, 2005; accepted August 4, 2005.

BACKGROUND. Advances in imaging technology have enhanced the detection of small nodular lesions during the course of chronic liver disease.

METHODS. Between 1995 and 2002, the authors examined 154 consecutive patients with small hepatic nodules without hepatocellular carcinoma (HCC) over a median duration of 2.8 years. The median size of these nodules was 14 mm (range, 7–40 mm). The initial histopathologic diagnosis included high-grade dysplastic nodule (HGDN) (n = 13), low-grade dysplastic nodule (LGDN) (n = 42), and regenerative nodule (RN) (n = 99).

RESULTS. A total of 29 (18.8%) nodules developed into HCC during the observation period. Cumulative HCC development rates at the first, third, and fifth year were 46.2%, 61.5%, and 80.8% for HGDN; 2.6%, 30.2%, and 36.6% for LGDN; and 3.3%, 9.7%, and 12.4% for RN, respectively. The rate of HCC development was significantly higher in the HGDN group than for other types (P < 0.001). Multivariate analysis disclosed that histopathologic diagnosis (P < 0.001) and findings on computed tomographic arterial portography (CT-AP) (P = 0.004) were significantly associated with future HCC development. The hazard ratios of HGDN and LGDN were 16.8 (95% confidence interval [CI], 6.19–45.6) and 2.96 (95% CI, 1.20–7.31), respectively. A decrease in portal blood flow also showed a significantly high hazard ratio of 3.04 (95% CI, 1.42–6.50). Approximate annual development rate to HCC was 20% in patients with HGDN and 10% in LGDN.

CONCLUSION. HGDN should be considered a precancerous lesion when it appears during follow-up of chronic viral hepatitis or cirrhosis. Reduced portal blood flow in the nodule on computed tomography-AP is also an important predictor for development of hepatocellular carcinoma. Cancer 2006;106:636–47.

© 2005 American Cancer Society.

KEYWORDS: hepatocellular carcinoma, dysplastic nodule, multistep carcinogenesis.

With advances in imaging diagnosis, hepatic nodular lesions are now frequently found during the course of chronic liver disease.1–6 According to a previous study, hepatocellular carcinoma (HCC) develops at a rate of about 3–10% per year in patients with chronic viral hepatitis and cirrhosis.7–10

If an intrahepatic nodular lesion is found, it is always necessary to consider development of HCC. Screening for HCC is mostly performed by ultrasonography (US). If hepatic nodular lesions are found on US, further imaging diagnosis such as computed tomography (CT), magnetic resonance imaging (MRI), and hepatic angiography are performed to decide if the nodules represent HCC. Fine needle aspiration biopsies are considered only when HCC is not diagnosed by
such imaging techniques, because careless needle insertion into HCC may lead to tumor seeding to the surrounding liver tissue.\textsuperscript{11,12}

Some hepatic nodules are diagnosed as HCC by both imaging and biopsy examination and can be treated with appropriate modalities, whereas others are diagnosed as nonmalignant. During the observation of such “nonmalignant” hepatic nodules, some progress to HCC while others disappear or stay unchanged for long periods.

It is generally considered that there are two different pathways to the development of HCC.\textsuperscript{13,14} One is a “multistep” carcinogenesis process similar to the process described for colorectal cancer,\textsuperscript{15,16} and the other is called “de novo” carcinogenesis. With the former type, it is important to determine which types of hepatic nodules are precancerous. In other words, identification of risk factors for development of HCC is necessary for follow-up of such hepatic nodules.

The aim of the present study was to elucidate outcomes and factors associated with development of HCC during long-term follow-up of small hepatic nodules that were initially diagnosed as nonmalignant lesions.

**MATERIALS AND METHODS**

**Patients**

Medical records of patients who were hospitalized for evaluation of hepatic nodular lesions at Toranomon Hospital between 1995 and 2002 were reviewed retrospectively. A total of 1425 patients were hospitalized during this period, and 1171 (82.2%) of the 1425 patients were already diagnosed as HCC by detailed image analysis. Fine needle aspiration biopsies were carried out in the remaining 254 (17.8%) patients and 88 (34.6%) were histologically diagnosed as HCC. After exclusion of hepatic hemangiomas (n = 4), focal nodular hyperplasia (n = 3), and alcoholic hyperplastic nodules (n = 5), the remaining 154 patients who had a histologic diagnosis, but were not diagnosed as HCC, were enrolled in the current study. If two or more hepatic nodules were observed in the same patient, then the largest nodule was included in the current study. Clinical backgrounds of these patients are listed in Table 1. The background of all 154 patients included chronic liver disease as 108 (70.1%) patients were positive for hepatitis C virus (HCV), 32 (20.8%) patients were positive for hepatitis B virus (HBV), and 2 (1.3%) patients were positive for both HCV and HBV. Twelve (7.8%) of 154 were negative for both HCV and HBV. Among these 12 patients, only 4 (33.3%) had a history of habitual heavy alcohol intake of more than 80 g per day. In addition, cirrhosis was evident in 124 (80.5%) patients. In our institution, informed consent is not required for reviewing patient records, including images, and, therefore, no such consent was obtained. However, at the time of the study, each patient provided informed consent for conducting imaging studies, including dye injection and needle biopsy.

**Image Analysis**

US or helical dynamic CT was carried out every 3 months for follow-up and examined for a change in imaging findings. US examination was generally performed with B-mode fundamental and harmonic imaging. Contrast-enhanced US using Levovist (Scheriing, Berlin, Germany) was performed in some cases. Dynamic CT scans were performed using a single-detector helical CT scanner (Hi-Speed advantage SG, GE Yokogawa Medical Systems, Tokyo, Japan). Furthermore, all CT scans throughout the study were performed using this scanner. In these studies, 95 mL of 350 mg I/mL Iomeporl (Iomeron 350, Eisai, Tokyo), as the contrast medium, was rapidly injected intravenously at 0.06 mL/kg body weight/sec. Phase-1, -2, and -3 imaging were performed at 25, 60, and 180 seconds (slice thickness: 10 mm, 5 mm, and 10 mm, respectively) after the start of injection, respectively.

The radiologic study was conducted by intraarterial digital subtraction angiography, including celiac and mesenteric angiography and selective angiography of the common hepatic artery. Computed tomographic arterial portography (CT-AP) and computed tomographic hepatic angiography (CT-HA) were carried out in the CT room after completion of hepatic

### Table 1

| Clinical Background of 154 Patients with Small Hepatic Nodular Lesions |
|---------------------|------------------|
| Characteristic      | Median (range)   |
| Age in yrs          | 63 (33–81)       |
| Gender, male:female | 101:53           |
| HBV:HCV:HBV+HCV:others | 32:108:2:12     |
| Previous history of HCC, yes:no | 40:114          |
| Presence of cirrhosis, yes:no | 124:30         |
| Diameter of nodule, mm | 14 (7–40)       |
| Albumin, g/dL       | 3.7 (2.6–4.7)    |
| Bilirubin, mg/dL    | 1.0 (0.4–3.1)    |
| ICG R15, %          | 27 (4–79)        |
| Platelet, $\times 10^4$/mm$^3$ | 10.2 (3.4–38.0) |
| Prothrombin time, % | 88 (50–100)      |
| AFP, ng/mL          | 13 (1–1070)      |
| DCP, AU/mL          | 13 (<10–182)     |
| Observation periods, yrs | 2.8 (1.8–6.9) |

\(\text{HBV: hepatitis B virus; HCV: hepatitis C virus; HCC: hepatocellular carcinoma. ICG R15: indocyanine green retention rate at 15 minutes; AFP: alpha-fetoprotein; DCP: des-gamma-carboxy prothrombin.}
\(\text{Data are expressed as ratios, not median and range.}\)
angiography using 5-French catheter. CT-AP scans were carried out with slip-ring technology, 5-mm thick sections, and 5-mm collimation. Overlapping reconstructions were obtained every 2.5 mm. Data acquisition was started 25 seconds after initiation of a transcatether hepatic artery injection into the superior mesenteric artery of 90 mL of nonionic contrast material containing 120 mg I/mL at 3 mL/sec, by using an automated power injector. The duration of scanning was around 25–35 seconds, depending on liver size, during a single breath hold. CT-HA scans were also obtained with 5-mm thick sections, 5-mm collimation, 2.5-mm reconstruction intervals. Data acquisition was started 10 seconds after initiation of a transcatheter hepatic artery injection (using the same automated power injector) of 20–30 mL of nonionic contrast material that contained 70 mg I/mL at 1.0 mL/sec.

Both CT-AP and CT-HA were qualitatively analyzed retrospectively, and the final diagnosis was established by consensus between two experienced radiologists who were blinded to the histologic diagnosis and clinical outcome. The CT-AP findings in hepatic nodules were classified as isoattenuating or low-attenuating, compared with the surrounding liver parenchyma. If only part of the nodule was hypodense, then we defined such nodule as low-attenuating. Likewise, CT-HA findings were classified as isoattenuating or high-attenuating compared with surrounding liver parenchyma. Because we were interested in increased intranodular arterial blood flow in the current study, if the nodule was hypodense on CT-HA, it was regarded as isoattenuating.

Follow-Up Protocol
On the basis of the above-mentioned strategies, when the tumor diameter enlarged or there was a change in US pattern or change of enhancement features, reexamination, including imaging and tumor biopsy, was considered. When a typical hypervascular staining pattern was obtained on angiography or a hyperattenuating nodule was detected on the arterial phase of the dynamic CT, the nodule was diagnosed as HCC without histologic examination.

Histopathologic Examination
All specimens were obtained by percutaneous fine needle aspiration biopsy (FNAB) by using a 21-gauge Mashima needle under US guidance. Tissue samples were collected not only from tumor tissue but also from nontumor tissue to compare architectural and nuclear differences. To avoid misdiagnosis due to sampling error or variation, sampling was carried out at least twice from different areas of the nodule. Furthermore, the US image was recorded on a video recorder to confirm that tumor samples were correctly obtained. Sections of 3–6-μm in thickness were cut after formalin-fixation and paraffin-embedding of specimens. Sections were stained with hematoxylin and eosin (H & E) and silver for reticulin fibers.

Tissue samples obtained by tumor biopsy were classified into HCC, high-grade dysplastic nodule (HGDN), low-grade dysplastic nodule (LGDN), or regenerative nodule (RN), according to criteria proposed by an international working party. In brief, LGDN was characterized by a slight increase (<1.5 times) of cell density and nuclear-cytoplasmic ratio compared with the surrounding liver tissue, an absence of structural dysplasia, and sometimes showed a large or small change. HGDN also had features of LGDN, and, in addition, there was an increase in cell density of between 1.5 to 2 times, high nuclear-cytoplasmic ratio, cytoplasmic basophilia, and irregular nuclear contour. If stromal or portal tract invasion of the tumor was seen in the specimen, the nodule was considered well differentiated HCC.

Statistical Analysis
Differences in background features and laboratory data among the three groups were analyzed by the chi-square test and Kruskal–Wallis test. The time between first biopsy and development of HCC was analyzed by using the Kaplan–Meier technique, and differences in curves were tested by using the log-rank test. Independent risk factors associated with HCC progression rate were studied using stepwise Cox regression analysis. Potential risk factors for malignant transformation that were assessed included the following 18 variables: age, gender, etiology of background liver disease, previous history of HCC, presence of cirrhosis, albumin, bilirubin, indocyanine green retention rate at 15 minutes (ICG R15), platelet count, prothrombin time, alpha fetoprotein (AFP), des-gamma-carboxy prothrombin (DCP), diameter of the nodule, US pattern, conventional CT findings, CT-AP findings, and CT-HA findings. A probability of <0.05 was considered significant. Data analysis was performed using SPSS statistical software version 10 (SPSS Inc., Chicago, Illinois).

RESULTS
Image Diagnosis and Malignant Transformation of Hepatic Nodular Lesions
Each imaging technique and lesion feature were examined. Because all tissue samples in the current study were obtained under US guidance, the detection rate on US was 100%. Of hepatic lesions examined, 85 (55.2%) nodules were low-echoic whereas the remain-
ing 69 (44.8%) were high-echoic. Furthermore, 35 (22.7%) nodules were observed on helical dynamic CT. Among these, 26 were isoattenuating at arterial phase and low-attenuating at portal venous phase and/or the equilibrium phase. The other nine showed low attenuation throughout the scanning. None of the nodules was high-attenuating at the arterial phase on CT.

CT-AP was carried out in 144 patients and 49 (34.0%) nodules were detected. Among these, 30 nodules were slightly low attenuating, whereas the remaining 19 were markedly low attenuating relative to surrounding liver parenchyma. CT-HA was performed in 142 patients and 40 (28.2%) nodules were detected. Only 10 nodules were high attenuating, whereas 30 nodules were low attenuating on CT-HA.

The cumulative HCC development rate was evaluated by Kaplan–Meier method for each imaging diagnosis. The HCC development rates for high echoic nodules were 4.8% at 1 year, 13.7% at 3 years, and 22.5% at 5 years. These rates were 9.8%, 25.7%, and 25.7% for low-echoic nodules, respectively, and the rates were not significantly different between the high- and low-echoic groups. The HCC development rate of CT-detected and -undetected nodules was 9.7% and 4.3% at 1 year, 32.2% and 16.8% at 3 years, and 38.3% and 20.6% at 5 years, respectively. CT-detected nodules were more likely to transform to HCC than CT-undetected nodules (log-rank test, P < 0.039) (Fig. 1A). HCC developed from CT-AP low-attenuating nodules at a rate of 13.8%, 35.3%, and 39.7% at 1, 3, and 5 years, respectively. These rates were 4.7%, 14.5%, and 19.4%, respectively, for nodules that did not show low attenuation. The CT-AP low-attenuation nodules often transformed to HCC (log-rank test, P = 0.005) (Fig. 1B). Similarly, HCC developed from CT-HA high-attenuation nodules at a rate of 9.2%, 20.0%, and 30.5% at 1, 3, and 5 years, respectively. These rates were 5.7%, 13.8%, and 23.4%, respectively, for high-attenuation nodules, and the rates were not significantly different between the two groups.

**Histologic Diagnosis and Malignant Transformation of Hepatic Nodular Lesions**

Histologic diagnosis of 154 nodules based on examination of initial biopsies was as follows: HGDN, n = 13; LGDN, n = 42; whereas the remaining 99 nodules did not show any abnormal histologic features and were, therefore, considered RN. The clinical backgrounds of the three groups classified by histologic features are summarized in Table 2. There were no differences in these parameters among the three groups. Tumor diameter, US pattern, detection of the nodule on dynamic CT, and attenuation pattern on CT-AP and CT-HA were also compared (Table 3), but there were no significant differences among the three groups.

Twenty-nine (18.8%) patients of 154 with nodules progressed to HCC during the median follow-up of 2.8 years. The diagnosis of HCC was made histologically (n = 24) or by imaging modalities (n = 5). The 24 HCC that were histologically diagnosed comprised well differentiated HCC (n = 16) and moderately differentiated HCC (n = 8). The cumulative HCC progression rate was 7.0% at 1 year, 16.1% at 2 years, 19.9% at 3 years, and 24.4% at 5 years. The first histologic diagnosis and the final outcome of the 154 nodules at the end of the observation period are shown in Figure 2. A total of 9 (69.2%) of 13 nodules transformed to HCC.
from HGDN. Only one nodule disappeared during the follow-up period. Similarly, 11 (26.2%) nodules of 42 progressed to HCC from LGDN, and 5 (11.9%) nodules disappeared. A total of 9 (9.1%) HCC arose from RNs at first biopsy, and 37 (37.4%) disappeared.

The cumulative HCC development rate calculated by the Kaplan–Meier method was 7.0% at 1 year, 19.9% at 3 years, and 24.4% at 5 years. The development of HCC occurred within the first 5 years, and no patient has subsequently developed HCC to date. Figure 3 shows HCC development rate according to each tissue diagnosis. The HCC transformation rate from HGDN was 46.2% at 1 year, 61.5% at 2 and 3 years, and 80.8% at 5 years. The rate of development of HCC from LGDN was 2.6% at 1 year, 30.2% at 3 years, and 36.6% at 5 years. Similarly, HCC developed from RNs at a rate of 3.3% at 1 year, and 9.7% at 3 years, and 12.4% at 5 years. HCC developed more often from HGDN nodules than from LGDN and RN (log-rank test, \( P < 0.0001 \)).

**Predictive Factors for Development of HCC from Hepatic Nodular Lesions**

To elucidate predictive factors for development of HCC from hepatic lesions, both patient and tumor characteristics were analyzed by the log-rank test. Age > 60 years, ICG R15 > 30%, tumor diameter > 14 mm, detection of the nodule on conventional helical dynamic CT, decrease of portal blood flow in the hepatic
A nodule on CT-AP, and liver histology on tumor biopsy were significant factors by univariate analysis. Furthermore, the etiology of chronic liver disease, serum albumin, serum bilirubin, prothrombin time, serum AFP level, serum DCP level, platelet count, US pattern, and hyperattenuation on CT-HA were not significant.

Subsequently, multivariate analysis by the Cox proportional hazard model was performed to adjust for the confounding effect on each variable. Histologic diagnosis was the most significant factor for development of HCC (P < 0.0001). Compared with RN, the rate of development of HCC in HGDN was as much as 16.8-fold higher (95% confidence interval [CI], 6.19–45.6), and in LGDN was 2.96-fold (95% CI, 1.20–7.31) higher. Decreased portal blood flow in the nodule on CT-AP was also significant (hazard ratio [HR], 3.04; 95% CI, 1.42–6.50; P = 0.004) (Table 4).

In addition, HCC developed at other sites of the liver during observation in 49 patients. However, there was no correlation between malignant transformation of the observed nodule and the subsequent development of HCC in other sites. In addition, no HCC developed from smaller nodules that were identified on first examination, during the follow-up period.

**Case Reports of Hepatic Nodule Transforming to HCC during Four-Year Follow-Up**

Figure 4 shows a case of HCC that progressed from a dysplastic nodule. This patient was a 61-year-old male with hepatitis C virus (HCV)-related cirrhosis. A 6-mm diameter hyperechoic nodule was found on US during the course of cirrhosis (Fig. 4A). Although detailed imaging analysis, including dynamic CT, hepatic angiography, CT-HA, and CT-AP, was carried out, the nodule was not detected with these modalities. FNAB was performed under US guidance, and the histologic diagnosis obtained from the specimen was LGDN (Fig. 5A). Therefore, the nodule was carefully followed up every 3 months on US. Three years later, the nodule was a little enlarged to 9 mm in diameter, and low-echoic foci appeared inside the nodule (Fig. 4B). Another year later, diameter of the tumor rapidly increased to 17 mm, and the nodule showed a “mosaic pattern,” which is the typical sign of classical HCC on US (Fig. 4C). On this account, HCC development of the nodule was strongly suspected. Hepatic angiography was then carried out and showed typical hypervascular staining (Fig. 4D). The nodule was surgically resected afterward, and the specimen showed histologic features of moderately differentiated HCC (Fig. 5B).

Figure 6 shows another case of HCC that progressed from HGDN. This patient was a 65-year-old male with HCV-related cirrhosis. A 16-mm diameter hyperechoic nodule was found on US during the course of cirrhosis (Fig. 6A). The nodule was not detected on helical dynamic CT. Hepatic angiography, including CT-AP and CT-HA, was carried out, and a vague slightly low-attenuating area was detected on both CT-AP and CT-HA (Fig. 6B–C). FNAB was performed under US guidance, and the histologic diagnosis was HGDN (Fig. 7A). Two years later, the nodule was found slightly enlarged to 20 mm in diameter on US, and the nodule became detectable on dynamic CT (Fig. 6D). Furthermore, detailed image diagnosis was performed. Although there was no hypervascular staining on hepatic angiography, a relatively well bordered low-attenuating area was detected on CT-AP (Fig. 6D). The nodule was surgically resected afterward, and the specimen showed histologic features of moderately differentiated HCC.
A 6-mm diameter hyperechoic nodule appeared on ultrasonography (US) during the follow-up of HCV-related cirrhosis. Four years later, the nodule increased in size to 9 mm in diameter and low-echoic foci appeared inside the nodule. The nodule rapidly grew in size within 1 more year and showed a “mosaic pattern” on US. Hepatic angiography was carried out before surgical resection. The angiogram showed typical hypervascular staining of HCC in the right lobe of the liver.

FIGURE 4. (A) A 6-mm diameter hyperechoic nodule appeared on ultrasonography (US) during the follow-up of HCV-related cirrhosis. (B) Four years later, the nodule increased in size to 9 mm in diameter and low-echoic foci appeared inside the nodule. (C) The nodule rapidly grew in size within 1 more year and showed a “mosaic pattern” on US. (D) Hepatic angiography was carried out before surgical resection. The angiogram showed typical hypervascular staining of HCC in the right lobe of the liver.
specimen showed histologic features of well differentiated HCC (Fig. 7B).

Comparison of Tumor Diameter at First Biopsy and End of Observation Period

The above-mentioned analysis reviewed predictive factors for development of HCC by liver tissue diagnosis at the first tumor biopsy. When we observed a change in nodule diameter during follow-up of more than 5 mm, HCC developed in 10 of 17 patients, whereas HCC developed in only 12 of 137 nodules in which the diameter enlarged by less than 5 mm. The relation between tumor enlargement and HCC progression was statistically significant ($P < 0.001$).

DISCUSSION

The importance of dysplastic nodules as precancerous lesions of HCC is well established in Japan, but less emphasized in Western countries. Sakamoto et al. studied 320 resected liver tissues and concluded that multistep carcinogenesis is one pathway to HCC development. Furthermore, several reports from Western countries in explanted whole liver from non-Japanese patients suggested that macroregenerative nodules may also represent precancerous lesions.

However, consistent with previous reports, not all hepatic nodular lesions that we found on screening by US progressed to HCC. Some nodules remained unchanged, and other nodules disappeared during long-term observation. Therefore, identification of true precancerous liver lesions, especially among patients with chronic liver disease, is important. The aims of the current study were to estimate the HCC progression rate of hepatic nodular lesions and to examine factors associated with malignant transformation.

Several reports have examined HCC development from borderline lesions; however, no reports have included as many patients as the current study of 154 patients who were histologically diagnosed with dysplastic nodules and fully examined by imaging procedures before tumor biopsy. Notably, all patients also received imaging diagnosis every 3 months with a median follow-up of 2.8 years.

Twenty-nine of 154 (18.8%) hepatic nodules in our study transformed into HCC. The cumulative HCC development rates for such intrahepatic nodular lesions were 7.0% at 1 year, 19.9% at 3 years, and 27.4% at 5 years. Our findings are similar to those of Borzio et al.,28 who reported an HCC rate of 31% in 90 large regenerative and dysplastic nodules and Seki et al.,24 who reported HCC development in 12.1% of 33 dysplastic nodules measuring $<3$ cm diameter at diagnosis.

With respect to predictive factors for development of HCC, age $>60$ years, ICG R15 $>30\%$, tumor diameter $>14$ mm, detection of a nodule on dynamic CT, decrease of portal blood flow in the hepatic nodule on CT-AP, and liver histology of tumor biopsy were significant factors by univariate analysis. The severity of background liver disease also has been reported as an important risk factor for development of HCC. Therefore, high ICG R15 may affect potential HCC development. With respect to the effect of age, underlying liver disease would be expected to advance with
aging, and genetic mutation of oncogenes or tumor suppressor genes could occur at higher frequency with aging. We speculate that such alterations may generally increase risk of carcinogenesis with increasing age.

With respect to tumor diameter, it is intuitive that larger nodules could represent a more advanced stage in multistep carcinogenesis, and, thus, would progress to HCC more frequently than smaller ones. There is a problem of lead-time bias at this point. However, in our study, 133 of the 154 (86.4%) patients had undergone US screening before the first detection of hepatic nodules, and, thus, lead-time bias should have been minimized.

In multivariate analysis, histologic diagnosis and decrease of portal flow in CT-AP were independent factors for prediction of malignant transformation. In our study, we classified liver histology into three groups; HGDN, LGDN, and RN. According to this classification, as predicted, the progression rate of HCC from HGDN was significantly high, and the annual HCC development rate exceeded 30% in the first 2 years. The regression coefficient of HGDN in multivariate analysis was as much as 16.8 compared with RN. Therefore, we can conclude that HGDN was a true precancerous lesion of HCC.

Because of radiologic innovations, the relation between tumor progression and vascular supply of hepatic tumors is well documented. Hayashi et al.29 followed up dysplastic nodules detected on CT-HA and CT-AP and described how portal blood flow in the nodule gradually decreased with acquisition of malignant tumor features. These results are consistent with our results in that in both univariate and multivariate analyses, reduced portal blood flow was a risk factor for HCC development. We realize that CT-AP and CT-HA are rather invasive methods and cannot easily be conducted repeatedly. Dysplastic nodules or RNs are usually isovascular or hypovascular.29 In fact, in our patients, the arterial phase on dynamic CT was isoattenuating in 26 and low-attenuating in the remaining 9, and all 35 nodules were low-attenuating at portal venous phase and/or the equilibrium phase. Although detection of the nodule on dynamic CT was significant for predicting HCC transformation, as determined by univariate analysis, this was not significant by multivariate analysis. This could mean that CT-AP is superior to dynamic CT in detecting reduced portal blood flow and, hence, a better predictor of progression to HCC.

With respect to evaluation of tumor arterial blood flow, although we reviewed the results of CT-HA in our patients, we could not determine the relation between arterial blood flow and tumor progression. Hepatic arterial flow decreased in dysplastic nodules and in early stage HCC, and then it increased as lesions progressed to classic HCC.29 Such a two-phase arterial
flow change may have complicated our statistical analysis.

Previous studies showed the usefulness of MRI for characterization of hepatic nodular lesions.32–35 Earls et al.32 examined thin-section MRIs of explanted liver and reported that MRI depicted 41 of 42 (98%) hepatic nodular lesions, which included dysplastic nodules as well as HCC. Furthermore, Matsui et al.33 demonstrated that hyperplastic adenomatous nodules were hyperintense on T1-weighted spin-echo imaging and hypointense on T2-weighted spin-echo imaging, and both features were useful for the differentiation of such borderline lesions from HCC in the cirrhotic liver. Furthermore, other groups also indicated the superiority of dynamic contrast-enhanced MRI and ferumoxides-enhanced MRI relative to CT for diagnosis of small HCC.34,35 We also examined dynamic MRI in 48 patients and ferumoxides MRI in 25 patients. Because the number of these patients was small, we did not include MRI results in our analysis. Choi et al.34 indicated that ferumoxides-magnetic resonance imaging can be used instead of CT-AP and CT-HA, because both modalities have almost the same sensitivity and high specificity for diagnosis of HCC. Further studies are needed to confirm the usefulness of ferumoxides-MRI for diagnosis of malignant transformation of hepatic nodular lesions.

There are certain limitations in our study. First, tumor biopsy was usually carried out under US guidance, and, therefore, nodules that were not observed on US were not included in this study. In our clinical practice, we sometimes find hepatic nodules that show low attenuation on CT-HA and/or CT-AP, but they are barely observed on US. Tsuchiyama et al.31 examined repeated CT-HA and reported that 18.8% of small stained spots progressed to HCC during a mean follow-up of 29 months. Indeed, it is technically difficult to confirm such nodules histologically, and detailed investigation is required in the future.

Second, problems of sampling error and sampling variation are always inherent in this kind of examination. Indeed, nine patients in our study progressed to HCC despite histologic diagnosis of RN at first biopsy. In these cases, we cannot exclude sampling errors or sampling variation. However, to minimize such problems, we routinely recorded the scene of the US-guided biopsy on video recorder to confirm that the sample was actually from the nodule. In addition, it is possible that samples obtained by needle biopsy did not reflect the most malignant part of the nodule, particularly when the nodule was heterogeneous. On this account, we usually obtained samples from two or more parts of a nodule to prevent sampling variation. If a nodule showed a heterogeneous US pattern, we obtained samples from each part of the nodule.

We recognized limitations of biopsy diagnosis for predicting HCC progression, as nine patients whose initial histologic diagnosis was RN later developed HCC. For this reason, it is necessary to include imaging diagnostic techniques such as CT-HA or CT-AP to predict liver cancer development.

Our present results allow us to conclude that dysplastic nodules, in particular HGDN, are true precancerous lesions of HCC. Hemodynamic changes in these nodules predicted progression to HCC; however, angio-computed tomography is an invasive examination, and repeated studies are not feasible. Recently, it was reported that HCC and borderline lesions, like dysplastic or regenerative nodules, can be discriminated by Levovist contrast-enhanced US.36,37 Because commercial use of Levovist was not possible until 1999 in our country, we have not included this technique in the current study. We are very interested in whether contrast-enhanced harmonic US is useful in predicting HCC development from hepatic nodular lesions, as this examination is less invasive than CT-HA or CT-AP and does not require hospitalization.

On the basis of changes observed during follow-up, our results indicated that enlargement of tumor diameter was the most important factor suggesting malignant transformation. Changes in US pattern also indicated HCC development. Indeed, low-echoic foci appeared in the center of hyperechoic nodules preceding HCC diagnosis in four patients who developed HCC. This finding is consistent with multistep carcinogenesis in HCC.

REFERENCES

1. Baron RL, Oliver JH 3rd, Dodd GD 3rd, Nalesnik M, Holbert BL, Carr B. Hepatocellular carcinoma: evaluation with biphasic, contrast-enhanced, helical CT. Radiology. 1996;199:505–511.
2. Oliver JH, III, Baron RL, Federle MP, Rockette HE Jr. Detecting hepatocellular carcinoma: value of unenhanced or arterial phase CT imaging or both used in conjunction with conventional portal venous phase contrast-enhanced CT imaging. AJR Am J Roentgenol. 1996;167:71–77.
3. Hollett MD, Jeffrey RB Jr., Nino-Murcia M, Jorgensen MJ, Harris DP. Dual-phase helical CT of the liver: value of early hepatic arterial phase scans in the detection of small (1.5 cm) malignant hepatic neoplasms. AJR. 1995;164:879–884.
4. Larson RE, Semelka RC, Bagley AS, Molina PL, Brown ED, Lee JK. Hypervascular malignant liver lesions: comparison of various MR imaging pulse sequences and dynamic CT. Radiology. 1994;192:393–399.
5. Yamashita Y, Hatanaka Y, Yamamoto H, et al. Differential diagnosis of focal liver lesions: role of spin-echo and contrast-enhanced dynamic MR imaging. Radiology. 1994;193:59–65.
6. Peterson MS, Baron RL, Murakami T. Hepatic malignancies: usefulness of acquisition of multiple arterial and portal venous phase images at dynamic gadolinium-enhanced MR imaging. Radiology. 1996;201:337–345.

7. Ikeda K, Saitoh S, Koida I, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. Hepatology. 1993;18:47–53.

8. Kasahara A, Hayashi N, Mochizuki K, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. Hepatology. 1998;27:1394–1402.

9. Tsai JF, Jeng JE, Ho MS, et al. Effect of hepatitis C and B virus infection on risk of hepatocellular carcinoma: a prospective study. Br J Cancer. 1997;7:968–974.

10. Benvegnu L, Gios M, Boccato S, Alberti A. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. Gut. 2004;53:744–749.

11. Sakurai M, Okamura J, Seki K, Kuroda C. Needle tract implantation of hepatocellular carcinoma after percutaneous liver biopsy. Am J Surg Pathol. 1983;7:191–195.

12. Smith EH. Complications of percutaneous abdominal fine-needle biopsy. Review. Radiology. 1991;178:253–258.

13. Sakamoto M, Hirohashi S, Shimosato Y. Early stages of multistep hepatocarcinogenesis: adenomatous hyperplasia and early hepatocellular carcinoma. Hum Pathol. 1991;22:172–178.

14. Rogler CE, Chisari FV. Cellular and molecular mechanisms of hepatocarcinogenesis. Semin Liver Dis. 1992;12:265–278.

15. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990;61:759–767.

16. Cho KR, Vogelstein B. Genetic alterations in the adenoma-carcinoma sequence. Cancer. 1992;70(suppl 6):1727–1731.

17. International Working Party. terminology of nodular lesions of the liver. Hepatology. 1995;22:983–993.

18. Nakano M, Saito A, Yamamoto M, Doi M, Takasaki K. Stromal and blood vessel wall invasion in well-differentiated hepatocellular carcinoma. Liver. 1997;17:41–46.

19. Cox DR. Regression models and life tables. J R Stat Soc. 1972;34:187–220.

20. Takayama T, Makuchii M, Hirohashi S, et al. Malignant transformation of adenomatous hyperplasia to hepatocellular carcinoma. Lancet. 1990;336:1150–1153.

21. Nakamura Y, Terada T, Terasaki S, et al. “Atypical adenomatous hyperplasia” in liver cirrhosis: low-grade hepatocellular carcinoma or borderline lesion? Histopathology. 1990;17:27–35.

22. Eguchi A, Nakashima O, Okudaira S, Sugihara S, Kojiri M. Adenomatous hyperplasia in the vicinity of small hepatocellular carcinoma. Hepatology. 1992;15:843–848.

23. Terasaki S, Kaneko S, Kobayashi K, Nonomura A, Nakamura Y. Histological features predicting malignant transformation of nonmalignant hepatocellular nodules: a prospective study. Gastroenterology. 1998;115:1216–1222.

24. Seki S, Sakaguchi H, Kitada T, et al. Outcomes of dysplastic nodules in human cirrhotic liver: a clinicopathological study. Clin Cancer Res. 2000;6:3469–3473.

25. Ferrell L, Wright T, Lake J, Roberts J, Ascher N. Incidence and diagnostic features of macroregenerative nodules vs. small hepatocellular carcinoma in cirrhotic livers. Hepatology. 1992;16:1372–1381.

26. Theise ND, Schwartz M, Miller C, Thung SN. Macrogenenerative nodules and hepatocellular carcinoma in forty-four sequential adult liver explants with cirrhosis. Hepatology. 1992;16:949–955.

27. Hytiroglou P, Theise ND, Schwartz M, Mor E, Miller C, Thung SN. Macrogenenerative nodules in a series of adult cirrhotic liver explants: issues of classification and nomenclature. Hepatology. 1995;21:703–708.

28. Borzio M, Fargion S, Borzio F, et al. Impact of large regenerative, low grade and high grade dysplastic nodules in hepatocellular carcinoma development. J Hepatol. 2003;39:208–214.

29. Hayashi M, Matsu O, Ueda K, et al. Correlation between the blood supply and grade of malignancy of hepatocellular nodules associated with liver cirrhosis: evaluation by CT during intraarterial injection of contrast medium. AJR Am J Roentgenol. 1999;172:969–976.

30. Lim JH, Cho JM, Kim EY, Park CK. Dysplastic nodules in liver cirrhosis: evaluation of hemodynamics with CT during arterial portography and CT hepatic arteriography. Radiology. 2000;214:869–874.

31. Tsuchiya T, Terasaki S, Kaneko S, Kaji K, Kobayashi K, Matsu O. Tiny staining spots in liver cirrhosis associated with HCV infection observed by computed tomographic hepatic arteriography: follow-up study. J Gastroenterol. 2002;37:807–814.

32. Earls JP, Theise ND, Weinreb JC, et al. Dysplastic nodules and hepatocellular carcinoma: thin-section MR imaging of explanted cirrhotic livers with pathologic correlation. Radiology. 1996;1:207–214.

33. Matsui O, Kadoya M, Kameyama T, et al. Adenomatous hyperplastic nodules in the cirrhotic liver: differentiation from hepatocellular carcinoma with MR imaging. Radiology. 1989;173:123–126.

34. Choi D, Kim S, Lim J, et al. Preoperative detection of hepatocellular carcinoma: ferumoxides-enhanced MR imaging versus combined helical CT during arterial portography and CT hepatic arteriography. AJR Am J Roentgenol. 2001;176:475–482.

35. Bluemke DA, Paulson EK, Choi MA, DeSena S, Clavien PA. Detection of hepatic lesions in candidates for surgery: comparison of ferumoxides-enhanced MR imaging and dual-phase helical CT. AJR Am J Roentgenol. 2000;175:1653–1658.

36. Fracanzani AL, Burdick L, Borzio M, et al. Contrast-enhanced Doppler ultrasonography in the diagnosis of hepatocellular carcinoma and premalignant lesions in patients with cirrhosis. Hepatology. 2001;34:1109–1112.

37. Suzuki Y, Fujimoto Y, Hosoki Y, et al. Clinical utility of sequential imaging of hepatocellular carcinoma by contrast-enhanced power Doppler ultrasonography. Eur J Radiol. 2003;48:214–219.