

Pathologic Diagnosis of Early Hepatocellular Carcinoma: A Report of the International Consensus Group for Hepatocellular Neoplasia

International Consensus Group for Hepatocellular Neoplasia

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Advances in imaging techniques and establishment of surveillance protocols for high-risk populations have led to the detection of small hepatic nodules in patients with chronic liver diseases, particularly those with cirrhosis or chronic hepatitis caused by hepatitis B or C viruses. These nodules, comprising a broad range of diagnostic entities—some benign and some with malignant potential—are currently defined histologically, and their clinical management often depends on the ability to make a reliable histologic diagnosis.

Evidence accumulated in the last two decades strongly favors the existence of a sequence of events in hepatic nodules that precedes the emergence of hepatocellular carcinoma (HCC),¹⁻¹⁰ and these lesions are recognized as precursors of HCC. However, from the beginning of their recognition, there has been considerable confusion concerning nomenclature and diagnostic approaches to these hepatic nodules. To clarify these issues, an International Working Party (IWP) of the World Congresses of Gastroenterology proposed a consensus nomenclature and diagnostic criteria for hepatocellular nodular lesions in 1995.¹¹ The IWP classified nodular lesions found in

chronic liver disease into large regenerative nodule, low-grade dysplastic nodule (L-DN), high-grade dysplastic nodule (H-DN), and HCC; this nomenclature has been widely adopted. In addition, the IWP introduced the concept of dysplastic focus as a cluster of hepatocytes with features of early neoplasia (in particular small cell change or iron-free foci in a siderotic background) measuring less than 0.1 cm, and defined small HCC as a tumor measuring less than 2 cm.

More recent studies support the division of small HCC into two clinico-pathological groups that have been termed early HCC and progressed HCC. Early HCC has a vaguely nodular appearance and is well differentiated. Progressed HCC has a distinctly nodular pattern and is mostly moderately differentiated, often with evidence of microvascular invasion.¹² Early HCC has a longer time to recurrence and a higher 5-year survival rate compared with progressed HCC.¹³

Small lesions with malignant potential have only subtle differences from the surrounding parenchyma, making them difficult to assess reproducibly. Differences in the application of diagnostic criteria between Western and Eastern pathologists has been a persistent difficulty in research and clinical management of these lesions.¹⁴ In order to obtain a refined and up-to-date international consensus on the histopathologic diagnosis of nodular lesions, such as dysplastic nodules and early HCC, the International Consensus Group for Hepatocellular Neoplasia (ICGHN) was convened in April 2002 in Kurume, Japan. The group has met several times up to July 2007 under the auspices of the Laennec Liver Pathology Society. The ICGHN is currently comprised of 34 pathologists and two clinicians from 13 countries. It includes most members of the original IWP who are still active and all the participants from the first ICGHN meeting. This consensus document summarizes the results of our meetings.

Materials and Methods

Twenty-six resected cases of nodules from 23 patients with chronic hepatitis or cirrhosis caused by hepatitis B or

Abbreviations: GPC3, glypican-3; GS, Glutamine synthetase; HCC, hepatocellular carcinoma; H-DN, high-grade dysplastic nodule; HSP70, heat shock protein 70; ICGHN, The International Consensus Group for Hepatocellular Neoplasia; IWP, International Working Party; L-DN, low-grade dysplastic nodule.

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Received June 11, 2008; accepted October 16, 2008.

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Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.22709

Potential conflict of interest: Nothing to report.

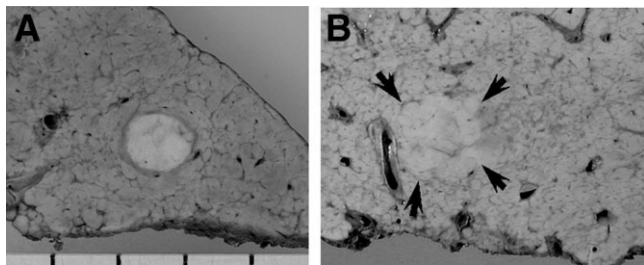


Fig. 1. (A) HCC of distinctly nodular type (progressed HCC), 12 mm in diameter. There was no discrepancy in the diagnosis of HCC with this growth pattern despite the small tumor size. (B) Small HCC of vaguely nodular type (early HCC) (arrows). These lesions were often a diagnostic problem, solved in part by recognition of the histologic features of stromal invasion.

C virus were selected from one Korean and two Japanese medical centers. All the lesions measured less than 2 cm in diameter. One hematoxylin and eosin–stained slide comprising the entire width of each lesion, a gross picture, and brief clinical data were reviewed by each pathologist individually, and the lesions were classified according to the IWP criteria. The group met at Kurume University Medical School, Kurume, Japan, in April 2002 to review all the lesions with photographs and by group review of relevant slides with a projecting microscope. The histologic diagnostic criteria were discussed, focusing on cases with marked discrepancies in initial, premeeting diagnosis. The second meeting was held at the University of Leuven, Belgium, in May 2004. The members discussed the diagnosis of an additional set of 22 resected small nodules. The third meeting was held at the Aristotle University of Thessaloniki, Greece in May 2006, and histopathologic consensus on both dysplastic nodules and early HCC was obtained. Kappa statistics were obtained from the comparative diagnostic panels of the first two of these meetings using SAS software version 8.2 (SAS Institute Inc., Cary, NC).

Summary of Comparative Diagnosis Data from Two Rounds of Slide Circulation

There was little difficulty in agreeing on the diagnosis of well-differentiated, small HCC of the distinctly nodular type or when the tumor was moderately differentiated HCC (Fig. 1A). The overwhelming diagnostic challenge was the differentiation of H-DNs from well-differentiated, small HCC of the vaguely nodular type (early HCC) (Fig. 1B). These lesions showed the lowest kappa value at the first conference with wide interobserver variation on initial review; the variation was diminished, but not totally resolved after the first conference. Initially, Asia-trained pathologists generally diagnosed HCC more frequently than Western pathologists. After the first con-

ference, this discrepancy decreased, and kappa values for HCC rose from 0.30 to 0.49 (though with different slide sets), with most Western pathologists ultimately agreeing with the diagnosis of HCC. The improvement of diagnostic agreement after the initial conference was due to the recognition of stromal invasion as a criterion for diagnosis of well-differentiated HCC. Stromal invasion is defined as tumor cell invasion into the portal tracts or fibrous septa within vaguely nodular lesions^{15,16} (Fig. 2).

Current Suggestions for Diagnostic Criteria

Gross and Radiographic Features

It is often possible to make a presumptive diagnosis of HCC when a small lesion is distinctly nodular and is hypervascular on contrast-enhanced imaging in the setting of cirrhosis.^{17,18} However, errors will occur occasionally with this approach. It has been reported that a small but significant proportion of explant livers was misdiagnosed as HCC.¹⁹ Any focal lesion containing a large arterio-venous shunt may be hypervascular (for example, focal nodular hyperplasia²⁰ or similar lesions²¹). A hypovascular lesion less than 2 cm having a vaguely nodular appearance cannot be accurately diagnosed by gross examination or imaging. Such lesions should undergo guided needle core biopsy.

Some small nodules have a “nodule-in-nodule” appearance either radiologically or on gross examination.²² In this situation, the subnodule usually represents dedifferentiation of the “parent” nodule. The parent nodule may be a dysplastic nodule or well-differentiated HCC, and the subnodule is invariably a less-differentiated lesion. In these situations, the entire nodule is classified by the worst component. Typically, the less-differentiated component is more vascular than the parent component.^{23–27} However, if the parent nodule is a dysplastic nodule and the subnodule is well-differentiated HCC, the subnodule may not be hypervascular, because unpaired arteries have not yet developed. Such unpaired arteries are small arteries (unaccompanied by bile duct) occurring

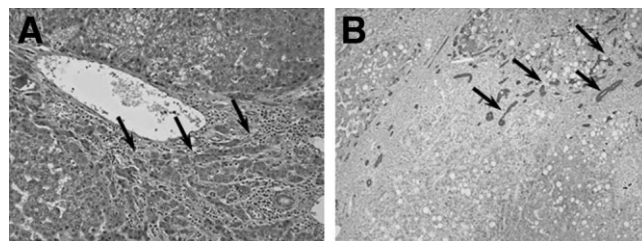


Fig. 2. (A) Stromal invasion in early HCC. The tumor cells (arrows) are invading an intratumoral portal tract. (B) CK19 immunostaining of another lesion. The ductular reaction (arrows) is mimicking stromal invasion and is prominent at the stroma-parenchymal interface. Well-differentiated HCC with fatty change is located at the bottom half of the image.

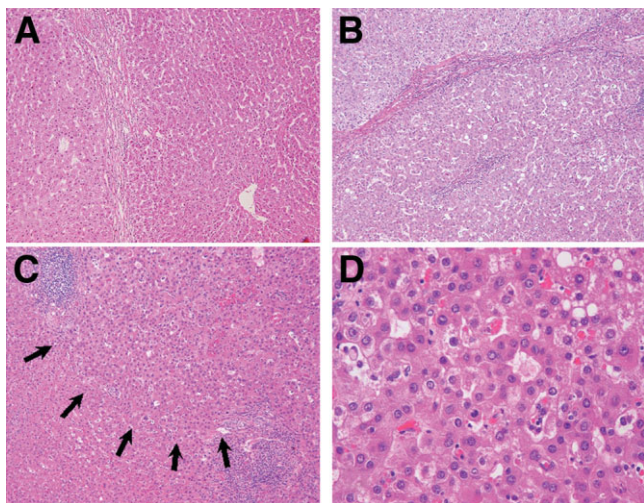


Fig. 3. (A) Low-grade dysplastic nodule (right two-thirds) shows mild increase in cell density with a clearer trabecular arrangement than the adjacent parenchyma. (B) High-grade dysplastic nodule. The cell density in this example is more than 1.5 times higher than that in the surrounding tissue (upper left). Irregularity of the trabecular pattern is remarkable, but there is no obvious infiltrative growth. (C, D) Small, well-differentiated HCC of vaguely nodular type (early HCC). The tumor shows replacing growth at the boundary (arrows), and the cell density is more than 2 times higher than that in the surrounding tissue. The tumor cells show an irregular thin-trabecular pattern with occasional pseudoglands. Stromal invasion was present elsewhere in the tumor.

outside the original portal tracts, and are indicative of neovascularization. They have a thin muscular wall that can be recognized in more detail via immunostaining for α -smooth muscle actin. Regardless of vascularity, a nodule-in-nodule appearance suggests the presence of HCC.

Pathologic Features

Low-Grade Dysplastic Nodules. L-DNs are sometimes vaguely nodular but are often distinct from the surrounding cirrhotic liver because of the presence of peripheral fibrous scar. This is not a true capsule, but rather condensation of scarring as is seen around all cirrhotic nodules. L-DNs show mild increase in cell density with a monotonous pattern, and they have no cytologic atypia, though they may have large cell change (formerly referred to as large cell dysplasia²⁸). Architectural changes beyond clearly regenerative features are not present; these lesions do not contain pseudoglands or markedly thickened trabeculae (Fig. 3). Unpaired arteries are sometimes present in small numbers.²⁹ Nodule-in-nodule lesions are not present in L-DNs. L-DNs may have diffuse siderosis or diffusely increased copper retention.

Among members of the consensus panels, there was no serious difficulty in differentiating L-DNs from early HCC. At the opposite end of the spectrum, distinction between L-DNs and large regenerative nodules was often

found to be difficult or impossible. Therefore, there is currently consensus that distinction between these two diagnostic categories cannot be made confidently by morphology alone and remains a task for the future. Fortunately, this distinction does not appear to have significant practical consequences at present.

High-Grade Dysplastic Nodules. H-DNs may be distinctly or vaguely nodular in the background of cirrhosis, although they also lack a true capsule, similar to L-DNs; however, they are more likely to show a vaguely nodular pattern than L-DNs. An H-DN is defined as having architectural and/or cytologic atypia, but the atypia is insufficient for a diagnosis of HCC. These lesions most often show increased cell density, sometimes more than 2 times higher than the surrounding nontumoral liver, often with an irregular trabecular pattern (Fig. 3). Small cell change (also known as small cell dysplasia³⁰) is the most frequently seen form of cytologic atypia in H-DNs. This form of atypia may also occur in small hepatocellular foci outside of H-DNs; the term dysplastic focus¹¹ may be appropriately used for such lesions. Large cell change may or may not be present in H-DNs. Unpaired arteries are found in most lesions, but usually not in great numbers. A nodule-in-nodule appearance is occasionally found in H-DNs, and subnodules often have a higher labeling index of Ki-67 or proliferating cell nuclear antigen than that of H-DN parenchyma. When a nodule with largely H-DN features contains a subnodule of HCC, the subnodule of HCC is usually well-differentiated with a well-defined margin.

The diagnostic discrepancy between H-DN and early HCC was frequent at the first consensus meeting, but was remarkably improved at the second meeting due to the recognition of stromal invasion as a diagnostic criterion for the differentiation of H-DN from early HCC. If areas of questionable invasion are present, immunostaining for keratins 7 or 19 may be useful; if such staining demonstrates a ductular reaction, the focus is considered a pseudoinvasion and does not warrant a diagnosis of HCC³¹ (Fig. 2B).

Early HCC (Small Well-Differentiated HCC of Vaguely Nodular Type)

Early HCC tumors are vaguely nodular and are characterized by various combinations of the following major histologic features^{6,22,32} (Fig. 3):

- (1) increased cell density more than 2 times that of the surrounding tissue, with an increased nuclear/cytoplasm ratio and irregular thin-trabecular pattern;
- (2) varying numbers of portal tracts within the nodule (intratumoral portal tracts);
- (3) pseudoglandular pattern;

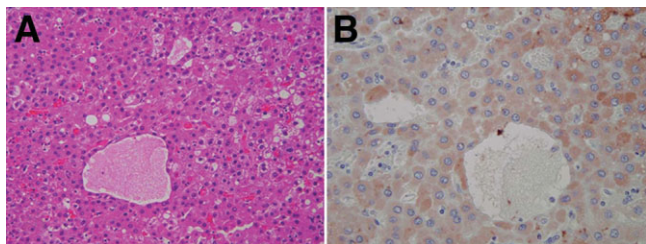


Fig. 4. GPC3 expression in small, well-differentiated HCC of vaguely nodular type (early HCC). (A) Hematoxylin-eosin stain. (B) Immunostaining for GPC3 shows expression in the cytoplasm of tumor cells.

- (4) diffuse fatty change; and
- (5) varying numbers of unpaired arteries.

Among these features, diffuse fatty change is observed in approximately 40% of cases.³³ The characteristic features of early HCC are sometimes seen in larger tumors as well—that is, well-differentiated tumors that measure over 2 cm and thus do not qualify for the designation of small HCC set forth by the IWP. The prevalence of fatty change decreases along with increasing tumor size; therefore, fatty change is uncommon in tumors larger than 3 cm. Fatty change is also uncommon in moderately differentiated HCCs. Any of the features listed above may be diffuse throughout the lesion or may be restricted to an expansile subnodule (nodule-in-nodule). Most importantly, because all of these features may also be found in H-DNs, it is important to note that stromal invasion remains most helpful in differentiating early HCC from H-DNs.

Emerging Tumor Markers

Alpha-fetoprotein is a well-established serum marker for HCC. However, elevated levels are rarely found in early HCCs. Alpha-fetoprotein is not useful as a tissue marker because of low sensitivity (25% to 30%), even with moderately differentiated HCC.

Glypican-3 (GPC3), a cell-surface heparan sulfate proteoglycans that is secreted into the plasma, has recently become established as a serum and tissue marker for HCC.³⁴⁻³⁹ GPC3 immunoreactivity has a reported sensitivity of 77% and specificity of 96% in the diagnosis of small HCC; therefore, GPC3 positivity is a strong argument for malignancy.^{40,41} The staining pattern is usually cytoplasmic but may be membranous or canalicular (Fig. 4). The monoclonal antibody from Biomosaics (IG12 clone) at a dilution of 1/50 to 1/100 as amplified with the new short polymer systems (Advance [Dako], Novolink [Novocastra], and Super-picture + [Zymed]) yields reliable results. Because GPC3 staining may be only focal, additional markers or a panel of markers may be necessary. GPC3 staining must be interpreted in context, be-

cause it may also be seen in regenerating hepatocytes in a setting of hepatitis⁴² and in melanocytic lesions.⁴³

Heat shock protein 70 (HSP70) belongs to a class of genes (heat shock proteins) implicated in the regulation of cell cycle progression, in apoptosis, and in tumorigenesis.⁴⁴⁻⁴⁶ Most HCCs are associated with chronic inflammation and fibrosis acting as stressful conditions that lead to heat shock protein synthesis. HSP70 is, in particular, a potent antiapoptotic survival factor. Chuma et al.⁴⁷ reported HSP70 as the most abundantly up-regulated gene among a set of 12,600 genes in early HCC. Furthermore, it was significantly overexpressed in progressed HCC as compared with early HCC, and in the latter as compared with precancerous lesions. HSP70 immunoreactivity was recently reported in the majority of HCCs, including early and well-differentiated forms, but not in nonmalignant nodules,⁴⁸ thus suggesting its use as a marker of malignancy. HSP70 immunoreactivity (SC-24, dilution 1:250 to 1:500 amplified with short polymer systems; Santa Cruz Biotechnology, Santa Cruz, CA) is nucleocytoplasmic and mostly focal with 70% sensitivity for HCC detection in surgically resected specimens.⁴⁹

Glutamine synthetase (GS) catalyzes the synthesis of glutamine from glutamate and ammonia in the mammalian liver⁴⁹ where it has been shown to be restricted to hepatocytes surrounding the terminal hepatic venules.⁵⁰ It is known that glutamine, the end product of GS activity, is the major energy source of tumor cells.⁵¹ Most importantly, GS is a target gene of β -catenin so that its overexpression is associated with mutations of β -catenin or with activation of this pathway.⁵²⁻⁵⁴ Up-regulation of GS messenger RNA, protein, and activity were shown by Christa et al.⁵² in human HCC, while Osada et al.⁵⁵ reported the stepwise increase in GS immunoreactivity from precancerous lesions to early HCC to progressed HCC. The monoclonal antibody from Chemicon International (clone MB302) at a dilution of 1/500 to 1/1000 and amplified with a new short polymer system yields reliable results. In order to increase its specificity as a marker of malignancy, GS immunostaining should be diffuse and of strong intensity, a pattern that can be seen in 50% of HCCs, including early forms.⁴⁹

The combination of more than one putative marker of malignancy raises the overall accuracy. When applying a panel of these three markers (GPC3, HSP70, and GS) to resected small lesions, the finding of any two positive markers had a sensitivity of 72% and a specificity of 100% to detect malignancy.⁴⁸ The diagnostic accuracy of this panel of markers in liver biopsies of hepatocellular nodules has not been yet tested.

Comment

The IWP criteria of 1995 have led to remarkable progress in global standardization of nomenclature of liver nodules.¹¹ However, although these criteria have been widely adopted, their application is challenging in equivocal lesions. Perhaps the most significant problem is that most histologic criteria are arrayed on a gradual spectrum and cannot be easily summarized as present or absent. Additionally, the number of criteria suggested in the literature are too numerous to achieve interobserver consensus, and the diagnostic weight carried by each of these criteria is uncertain. Frequently used criteria for malignancy in other tissues, such as mitotic activity and cellular atypia, are not represented to a significant degree in well-differentiated HCC. In addition, because the liver lacks a layered structure as seen in the gastrointestinal tract, it is difficult to determine the presence of destructive growth in early HCCs.

Despite these difficulties, current histologic criteria for these nodules clearly yield reliable diagnoses at both ends of the spectrum; most pathologists will correctly identify nodules up to L-DN as benign, whereas even small well-differentiated nodules with distinct nodular pattern or small moderately differentiated HCCs will be correctly identified as malignant. The remaining gray zone includes H-DN and early HCC. In evaluation of these lesions, the presence of stromal invasion is a useful criterion of malignancy.^{15,16} Accordingly, pathologists can decide whether the equivocal tumor is HCC or H-DN by recognizing the presence or absence of tumor cell invasion into the intratumoral portal tracts. When obvious stromal invasion is not found in an equivocal tumor, the lesion may be diagnosed as either H-DN or early HCC without detectable invasion. The diagnosis of stromal invasion is subjective and may require the assistance of histochemical (Victoria Blue or reticulin stains¹⁶) and immunohistochemical stains (keratin 7 or 19) for differentiation from pseudoinvasion.²⁹ New immunohistochemical and molecular markers are still under investigation and are likely to prove useful.^{46,54,56}

Role of Liver Biopsy. Regarding the application of biopsy for small nodules, the American Association for the Study of Liver Diseases recommends that biopsy should be performed for nodules less than 2 cm if their radiologic findings are not characteristic of HCC, whereas biopsy is not needed for lesions showing characteristic radiologic findings.¹⁷ This recommendation has been supported by prospective validation.¹⁸ Biopsy diagnosis of equivocal nodules remains a challenge, because minute biopsy specimens may not contain intratumoral portal tracts, thus precluding the detection of stromal invasion.

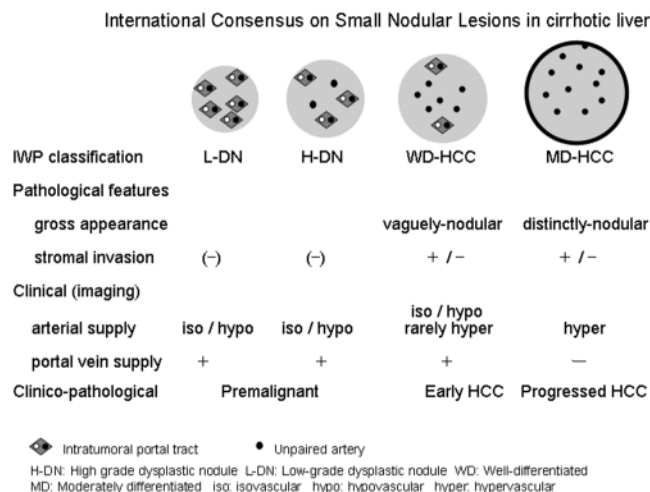


Fig. 5. Diagram summarizing clinical and pathological correlations. The cartoons in the top row show the anatomic changes that are found with the evolution of fully malignant HCC. Because early HCCs grow in a replacing pattern at the boundary, with tumor cells replacing the surrounding liver cell cords, they show a vaguely nodular appearance. When the tumors reach 1.5 to 2 cm in diameter, they tend to de-differentiate, becoming moderately differentiated and growing in an expansile fashion with formation of a fibrous capsule. Hypovascularity, hypervascularity, and isovascularity are understood to mean the signal intensity in the arterial phase of contrast-enhanced imaging relative to the nontumorous liver. Hypervascularity is related to the development of unpaired arteries, the absence of portal vein supply, and the distinctly nodular growth. The diagnosis must consider the context of the lesion, especially the presence of cirrhosis, the imaging findings, and the growth rate. In the appropriate context, a lesion with decreased portal vein supply without hypervascularity is suggestive of early HCC.

Similarly, the detection of unpaired arteries, mitoses, and various immunohistochemical markers are prone to sampling error. Core liver biopsy is definitely superior to fine needle aspiration, because the specimen obtained is suitable for the assessment of both architectural and cytologic features. Furthermore, the tissue block obtained provides materials for marker studies. Fine needle aspiration is usually adequate for the evaluation of large lesions that are likely to be moderately to poorly differentiated, where diagnostic criteria are easier to evaluate.

Clinico-pathological Correlation. Clinical and pathological features of early hepatocellular neoplasia are summarized in Fig. 5. The characteristic imaging appearance of HCC is a hypervascular lesion that shows washout in the portal venous phase. This appearance is also typical in small HCC of the distinctly nodular type and most moderately differentiated small HCCs. Dysplastic nodules and most early HCCs are hypovascular lesions. These classic images are explained by the anatomic features of the lesions. Taken together, the pathologic and imaging features define three phases in the evolution of neoplasia in cirrhotic liver, where dysplastic nodules represent the premalignant phase, well-differentiated HCC of the

vaguely nodular type represents early carcinoma, and small HCCs of the distinctly nodular type and moderately differentiated HCCs represent progressed carcinoma. In the noncirrhotic liver, however, the developmental process of HCC in humans has not been clarified.

Acknowledgment: The authors thank Mr. Kazuhiro Abe for kappa statistics.

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