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Invited Review

Analytical histopathological diagnosis of small hepatocellular nodules in chronic liver diseases

Y. Nakanuma[1], K. Hirata[1], S. Terasaki[2], K. Ueda[3] and O. Matsui[3]
Departmentments of [1]Pathology (II), [2]Internal Medicine (I) and
[3]Radiology, Kanazawa University School of Medicine, Kanazawa, Japan

Summary. Due to the recent progress in radiology and increased clinical and pathological interest, small hepatocellular nodules about 1 cm in size are frequently being detected in patients with chronic liver disease, particularly liver cirrhosis. Two new types of small hepatocellular nodules are now known: low-grade hepatocellular carcinomas (HCC) and dysplastic nodules, in addition to the previously known HCC (classical) and regenerative nodules. Ultrasound-guided needle biopsies from these nodules are routinely used for the differential diagnosis. For comparison, a simultaneous needle biopsy from the liver remote from the nodule is strongly recommended. Low-grade HCC, which are different from classical HCC in their morphological atypia and also biological behaviors, show local invasion into the portal tracts and surrounding hepatic parenchyma, but not intrahepatic or extrahepatic metastasis. Dysplastic nodules show mild cellular and structural atypia, a finding which is not sufficient for making a diagnosis of malignancy. An increased nuclear/cytoplasmic (N/C) ratio and nuclear crowding, small cell-dysplasia, increased cytoplasmic staining, clear cell change, pseudogland formation, and fatty change of hepatocytes are variably seen in these nodules. Nuclear changes, local invasion to the portal tract and surrounding liver, and loss of the reticulum fibers along the hepatocytes are useful markers favoring low-grade HCC rather than dysplastic nodules. These low-grade HCC and dysplastic nodules should also be distinguished from classical HCC as well as large-sized regenerative nodules. A comparative analysis of the histological findings observed in individual nodules is a reasonable approach to differential diagnosis at present. The recognition and analysis of these two new hepatocellular nodules may augur a new horizon in the study of hepatocellular neoplasm.

Offprint requests to: Dr. Yasuni Nakanuma, MD, Second Department of Pathology, Kanazawa University School of Medicine, Kanazawa 920-8640, Japan

Key words: Hepatocellular carcinoma, Low-grade hepatocellular carcinoma, Classical hepatocellular carcinoma, Dysplastic nodule, Large regenerative nodule

Abbreviations: HCC, hepatocellular carcinoma; N/C ratio, nuclear-cytoplasmic ratio

Introduction

Due to the rapid advances in imaging techniques for the liver and the careful clinical follow-up of patients with chronic advanced liver diseases (particularly viral and alcoholic liver cirrhosis), small nodular lesions about 1 cm in diameter are now frequently detected in such livers (Nakanuma et al., 1990, 1993; Matsui et al., 1991; Kondo, 1997; Okuda, 1997). These small heptocellular nodules are also increasingly recognizable in explants during liver transplantation (Ferrel et al., 1992; Theise et al., 1993). An important development in the study of hepatocellular neoplasm is the acceptance of two new hepatocellular nodules about 1 cm in diameter: dysplastic nodules (formerly known as borderline nodule, borderline lesion, adenomatous hyperplasia, atypical adenomatous hyperplasia, and macro-regenerative nodule type II) (International working party, 1995) and low-grade hepatocellular carcinoma (HCC) (also known as well-differentiated HCC). The term "low-grade HCC" is used in this review to denote a small-sized, well-differentiated HCC (Sugihara et al., 1992) without metastatic potential. While these small nodules are rather characteristic in their hemodynamics and imaging modalities at present, they are finally diagnosed histopathologically.

Although the identification and diagnosis of these small nodules in resected livers is becoming possible by experienced pathologists (Kondo, 1997), several difficulties and controversies remain regarding the differential diagnosis of such nodules in ultrasound-guided needle biopsy specimens (Sugihara et al., 1992; Nakanuma et al., 1993), as follows. First, limited

information from insufficient small specimens and sampling errors due to the histological heterogeneity within these nodules may lead to underdiagnosis or overdiagnosis. Second, regenerative reactive atypia of hepatocytes in chronic liver disease has not been studied thoroughly enough and the criteria for this atypia has not yet been accepted. These reactive changes in regenerating livers and tumor-like lesions resemble several atypical changes in dysplastic nodules or low grade-HCC (Nakanuma and Hirata, 1993; Kondo, 1997). It should be realized that dysplastic nodules do not always progress to low-grade HCC or classical HCC and some may regress: some low-grade HCC progress to classical HCC while some remain at a constant size for a considerable length of time (Takayama et al., 1990; Lencioni et al, 1994; Terasaki et al. in submission).

In this review, we will briefly describe the recently observed histopathological findings characterizing these small hepatocellular nodules, with an emphasis on differential diagnosis. We also shortly review the reactive atypical changes in hepatic parenchyma, and describe our routine experience of differential diagnosis using needle biopsy specimens from small nodules. In this review, hepatocellular nodules developing in non-cirrhotic portal fibrosis (idiopathic portal hypertension), metabolic liver diseases and chronic congestive livers, and liver cell adenoma and tumor-like lesions such as focal nodular hyperplasia and nodular regenerative hyperplasia will not be described.

Histological characteristics of small hepatocellular nodules

The types of small hepatocellular nodules about 1 cm in size which are frequently encountered in chronic advanced liver diseases, particularly viral and alcoholic cirrhosis, are listed in Table 1. They are largely dividable into neoplastic and non-neoplastic hepatocellular nodules.

I. Neoplastic hepatocellular nodules in chronic liver disease

A. Classical HCC

Also known as high-grade HCC, overt HCC.

Classical HCC has been described in many textbooks and is further classifiable histologically into trabecular, compact, pseudoglandular, pleomorphic, and scirrhous types (Fig. 1). Solitary small classical HCC about 1 cm in diameter are also occasionally encountered (Nakanuma et al., 1986; Kondo, 1997). They frequently show intra- and extrahepatic metastasis and portal venous emboli. They are frequently encapsulated fibrously.

B. Low-grade HCC

Also known as well-differentiated HCC, extremely well-differentiated HCC, early HCC, Edmondson's grade I HCC

The small-sized well-differentiated hepatocellular neoplasms show mild cellular and structural atypia as well as local invasive characters (Fig. 2A,B). They show

Table 1. Types of small hepatocellular nodules about 1 cm in size encountered in chronic advanced liver diseases.

Neoplastic hepatocellular nodules
Classical hepatocellular carcinoma
Low-grade hepatocellular carcinoma
Dysplastic nodule
Non-neoplastic hepatocellular nodules
Large regenerative nodule with unusual foci
Large regenerative nodule witout unusual foci
Anoxic pseudoglobular cirrhosis

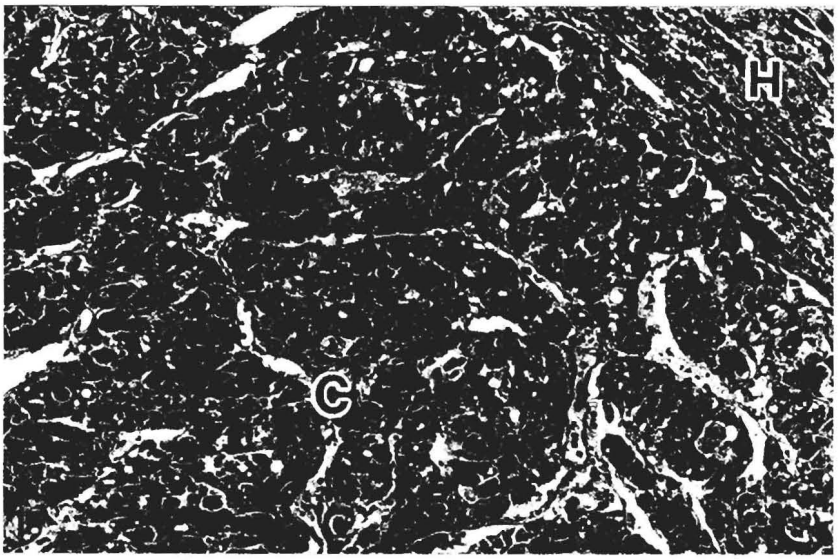

Fig. 1. Classical hepatocellular carcinoma (C) showing a trabecular/sinusoidal pattern and high grade atypia (surgically resected case). H: non-neoplastic hepatic tissue (chronic hepatitis C with cirrhosis). H&E, x 300

thin trabecular and compact (and to a lesser degree, pseudoglandular) patterns. Trabecular patterns with dense fibrous stroma are occasionally encountered. In addition, they show replacing growth or compressing growth against adjoining hepatocytes (Nakashima et al., 1982) and also a peculiar invasion into the portal tracts or fibrous septa (Nakano et al., 1997), while they do not have the ability to metastasize in the liver or outside the liver. While vague invasion into the small veins within the nodules is occasionally encountered, carcinoma emboli in the intrahepatic veins outside the nodule are not seen. Fibrous encapsulation is usually absent.

The natural course of this type of HCC remains unsettled (Arakawa et al., 1986; Takayama et al., 1990; Sakamoto et al., 1991; Lencioni et al., 1994): some low-grade HCC may progress to classical HCC, while others remain at a constant size for a longer period or may regress.

C. Dysplastic nodules

Also known as adenomatous hyperplasia, borderline lesion, borderline hepatocellular nodule, atypical adenomatous hyperplasia, equivocal malig-nancy, macroregenerative nodule type II.

This term, "dysplastic nodules", was coined by an International Working Party (1995) and is now becoming accepted, though the concept of this nodule is still confused and the diagnostic histological criteria may be different among histopathologists.

Dysplastic nodules are speculated to be neoplastic because they display variable atypical changes of hepatocytes (Fig. 3) and have an increased cell proliferative activity (Terasaki et al., 1991). It is of interest

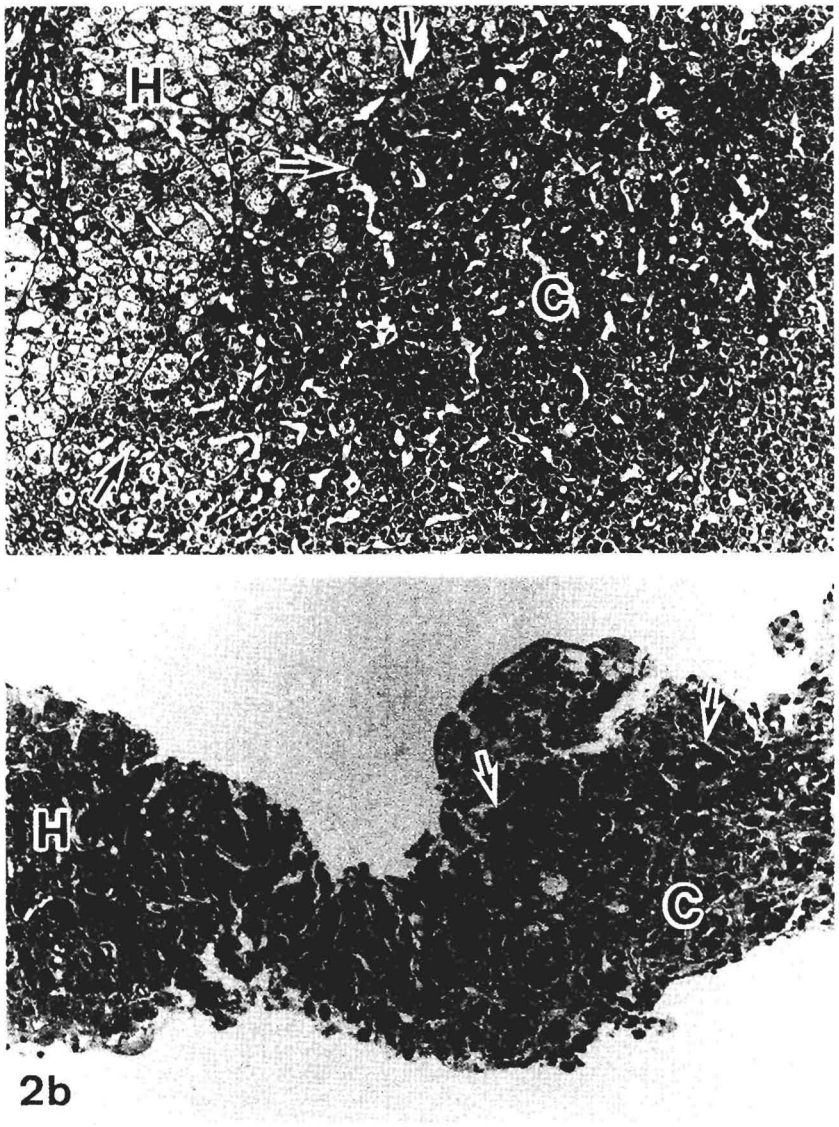

Fig. 2. A. Low-grade hepatocellular carcinoma (surgically resected case). Carcinoma tissue (C) is composed of small and hyperchromatic hepatoctes with a thin trabecular pattern and increased cellularity, and shows a replacing growth (arrows) to the surrounding liver (H) (chronic hepatitis C with cirrhosis). H&E, x250. **B.** Needle biopsy case of low-grade hepatocelllular carcinoma. Carcinoma tissue (C) shows an increased cellularity and several pseudogland formation (arrow). These histological features are clear when compared to the adjacent non-neoplastic hepatic tissue (H) (chronic hepatitis C with cirrhosis). H&E, x 300

that cancerous foci of low-grade HCC are occasionally encountered within this nodule (nodule in nodule lesions) (Arakawa et al., 1986; Sakamoto et al., 1991).

Although dysplastic nodules may be further divided into high-grade and low-grade nodules (Theise et al., 1993), histopathological definition, identification and significance of the low-grade dysplastic nodules remain unclear in the practial field. These nodules can be included in the group of regenerative nodule with unusual histological changes (see below).

II. Nonneoplastic hepatocellular nodules

These nodules of about 1 cm in size contain several small portal tracts and drainage veins. While the hepatocytes of these nodules are somewhat hyperplastic, the histopathologies of these nodules are essentially not different from the surrounding small-sized regenerative nodules or hepatic lobules (Fig. 4) (Furuya et al., 1988; Nakanuma et al., 1993). Careful histopathological studies have failed to detect malignant foci in these nodules, and follow-up studies of this type of nodule did not demonstrate malignant transformation. The majority of the nodules are simply a large-sized regenerative nodule, and a few may be related to the direct drainage of an aberrant gastric vein to some part of the liver (Matsui et al., 1995).

A. Large regenerative nodules with unusual lesions.

Also known as macroregenerative nodule type I, ordinary adenomatous hyperplasia, large regenerative nodule.

Various changes of the cellular and structural atypia

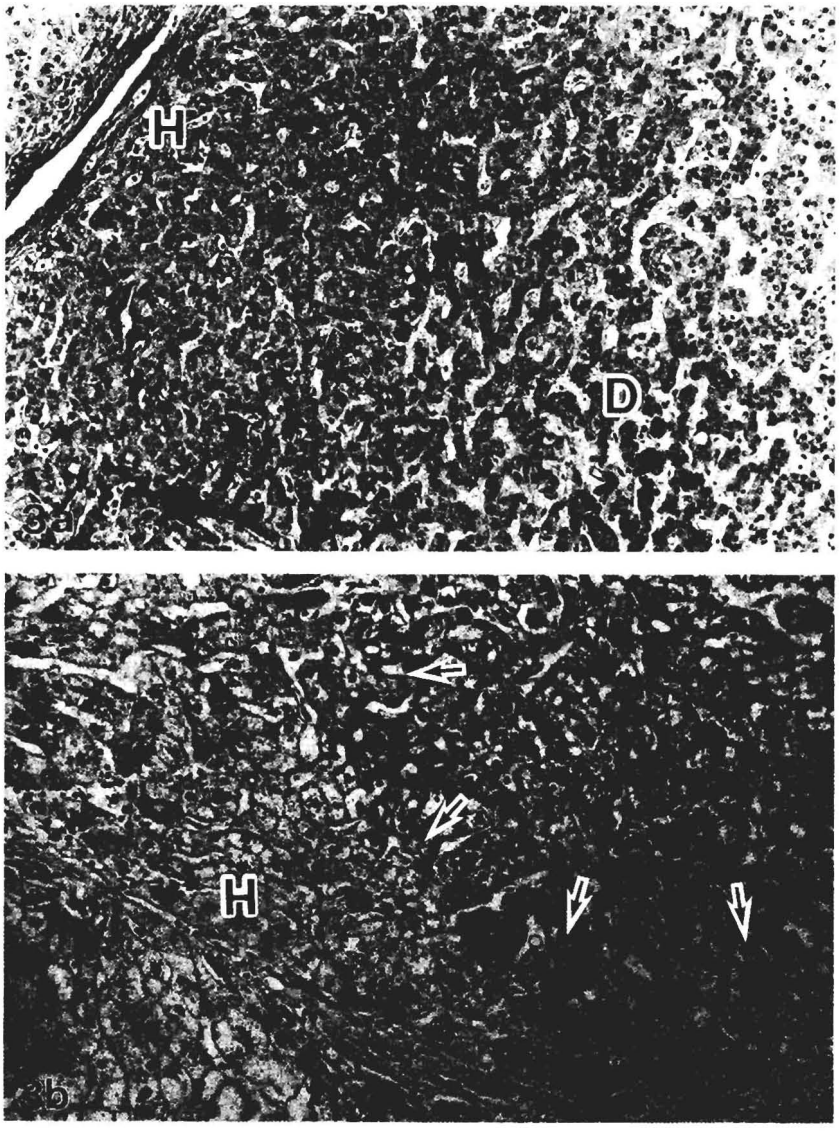

Fig. 3. A. A dysplastic nodule (D) is composed of small hepatocytes with hyperchromasia and has a thin trabecular pattern (surgically resected case). A few pseudogland formations are also seen. These changes are clear when compared to the adjacent hepatic tissue (H) (chronic hepatitis C with cirrhosis). H&E, x 250. **B.** A dysplastic nodule showing a thin-trabecular pattern and slightly hyperchromatic hepatocytes shows a vague replacing growth (arrows) to the surrounding liver (H) (chronic hepatitis C). H&E, x 320

described above in the dysplastic nodules are found in large regenerative nodules: their degree and distribution are mild and focal, respectively. These changes include Mallory body clustering, mild nuclear and cellular pleomorphism, perisinusoidal nuclear deviation, increased cytoplasmic staining, and fatty change.

It is difficult to differentiate this nodule from a dysplastic nodule in needle-biopsied small tissues.

B. Large regenerative nodules without unusual lesions

Also known as macroregenerative nodule type I, ordinary adenomatous hyperplasia, large regenerative nodule.

These nodules do not contain unusual findings within the nodules and are histologically similar to the surrounding hepatic parenchyma.

C. Anoxic pseudolobular necrosis

This lesion is usually an incidental postmortem finding, and was originally described by Edmondson (1967). This nodule is mainly a coagulative necrotic regenerative nodule, and is surrounded by zones of hemorrhagic and/or granulation tissue reaction. This lesion is occasionally detectable by imaging modalities in the clinical setting (Fukui et al., 1992). Surrounding cirrhotic nodules may also show similar necrotic changes at autopsy.

III. Histological changes useful for the identification and differential diagnosis of small hepatocellular nodules

Although the diagnosis of some of these nodules (particularly small-sized classical HCC) is easy, that of the remaining lesions, particularly low-grade HCC, dysplastic nodules and large regenerative nodules, is still a difficult task for histopathologists.

The following histological changes are seen in small hepatocellular nodules in variable degrees, frequency, extent and combinations (Ojanguren et al., 1997). In fact, the presence of such changes in the biopsy core may imply the exact hit of the biopsy needle to the nodule in the liver. Some of these findings are useful for the identification of nodular lesions but are of limited use for a differential diagnosis, while others are also valuable for making a differential diagnosis. It should be mentioned that these changes can be focally seen in renegenerative nodules or hepatic lobules, particularly around classical HCC (see the section of "Microscopic atypical foci in nonneoplatic parenchyma in chronic liver diseases" below).

A. Structural changes

The following findings are useful in the evaluation of structural changes of the nodules in chronic liver diseases.

Polarity of hepatic parenchyma to portal tracts and fibrous septa. This is well preserved in large regenerative nodules, slightly disturbed in dysplastic nodules, and vague and lost in low-grade and classical HCC, respectively. For example, continuity to the bile ductules in portal tracts to periportal hepatocytes is constantly recognizable in regenerative nodules and occasionally in dysplastic nodules, but vague and lost in low-grade HCC and classical HCC (Terada et al., 1995), respectively. Immunostaining of biliary cytokeratin is valid in the identification of biliary elements. A preferential deposition of copper granules (Nakanuma et al., 1979) or Mallory bodies in periportal or periseptal hepatocytes, when detectable, shows a similar

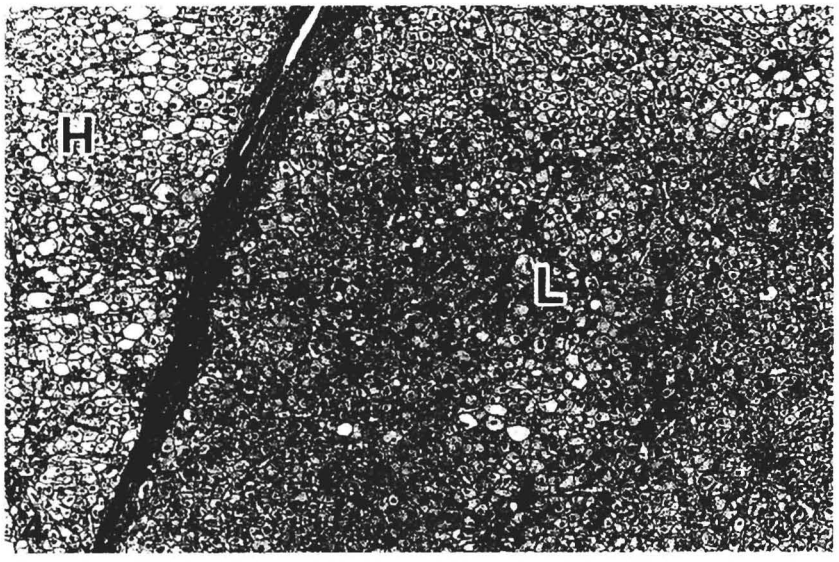

Fig. 4. A nodule (large regenerative nodule) (L) is composed of slightly hyperplastic hepatocyets which are similar to those of the surrounding hepatic tissue (chronic hepatitis C with cirrhosis) (H). Chronic hepatitis C with cirrhosis. H&E, x 250

distribution (Terada et al., 1989).

Portal tracts. Portal tracts are regularly and irregularly distributed in large regenerative nodules and in dysplastic nodules, while portal tracts are only occasional and immature or lost in low-grade HCC and lost in classical HCC.

Abnormal muscular vessels. These are frequently and considerably present in classical HCC and low-grade HCC, but are infrequent or inconspicuous in dysplastic nodules and only occasionally seen in regenerative nodules (Nakanuma et al., 1990).

B. Metastasis and local invasion

Metastasis. While classical HCC show intrahepatic or extrahepatic metastasis, low-grade HCC, dysplastic nodules, and large regenerative nodules do not.

Portal tract or stroma invasion. Foci of atypical hepatocytes appear to invade in enlarged portal tracts or fibrous septa (Nakano et al., 1997): some are isolated while others are continuous with periportal hepatocytes. The latter type resembles piecemeal necrosis or other types of destruction of limiting plates, while the former may resemble the entrapped hepatocytes in enlarged portal tracts as seen in chronic active hepatitis. The isolation of bile ducts within the neoplastic hepatocytes may also correspond to the extension of this lesion. These lesions are not infrequently seen in low-grade HCC and at a lesser frequency and degree in dysplastic nodules, while these lesions are not seen in classical HCC.

Replacing growth of hepatocytes. This is characterized by a direct growth of hepatocytes into the cord of adjacent normal hepatocytes which seem to replace but not destroy the latter (Nakashima et al., 1982). This is frequently seen in low-grade HCC (Fig. 2A) and classical HCC and also to a milder degree in dysplastic nodules (Fig. 2B). This creates a mosaic pattern or zigzag arrangement of neoplastic hepatocytes against the adjoining non-neoplastic hepatic parenchyma (Matsui et al., 1991). A similar growth of regenerating hepatocytes is also seen within actively regenerating non-neoplastic hepatic parenchyma.

Venous invasion. This is not seen in dysplastic nodules but is occasionally encountered within the nodules of low-grade HCC, while venous invasion or carcinoma emboli are frequently seen in the non-neoplastic hepatic tissue outside the carcinoma nodules in classical HCC. In the former, it is not infrequently difficult to distinguish this lesion from hepatocytes adjacent to dilated sinusoids or portal veins.

C. Cell structural changes

Multicell thickness of hepatocytes. This (more than 3 cell thickness) can be seen in classical HCC and low-grade HCC, while similar lesions are also focally seen in dysplastic nodules and also large regenerative nodules. A thin trabecular pattern (two to three cell thickness) is also frequently seen in dysplastic nodules and low-grade HCC (Kondo, 1997).

Microacinus or pseudogland formation. Dilated secondary canaliculi surrounded by more than 4 hepatocytes are regarded as this change. Microacinus formation refers to a more canalicular dilatation of trabeculae, while a pseudogland formation is a large and isolated lesion. These changes may produce an inspissated bile plug. Microacinus formation is more common in dysplastic nodules, while large-sized pseudoglands or a

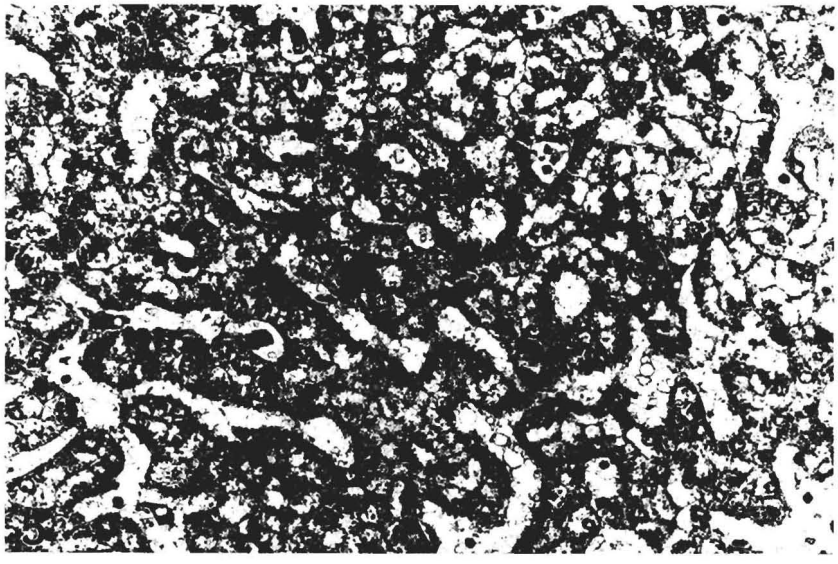

Fig. 5. This atypical area found in a large regenerative nodule with unusual lesion shows thin trabecular hepatocytes with clear cytoplasm and perisinusoidal nuclear deviation. Chronic hepatitis C with cirrhosis. H&E, x 320

cluster of pseudoglands in non-cholestatic livers may suggest low grade HCC (Fig. 2B) or classical HCC (pseudogland type).

Perisinusoidal distribution of nuclei. The hepatocyes presenting with this change show clear cytoplasm. The nuclei of adjoining hepatocytes in the trabecule regularly face the sinusoids. This lesion itself is seen in regenerative (Fig. 5) and dysplastic nodules and low-grade and classical HCC.

D. Nuclear changes

Nuclear mitoses are not a useful differential marker in this case, because they are rarely seen in dysplastic nodules or low-grade HCC. In dysplastic nodules and low-grade HCC, the nuclear sizes are rather uniform.

Anisonucleosis. This change with hyperchromasia implies classical HCC rather than low grade HCC. Large cell dysplasia also shows this change.

Increased nuclear/cytoplasmic (N/C) ratio with hyperchromasia. This may be seen in small, normal-sized or large hepatocytes, and can lead to the identification of nodules. Nuclear membrane thickening and an irregular nuclear contour are also differential markers suggestive of low-grade HCC rather than dysplastic nodules. In addition to these nuclear changes, other changes are required for a differential diagnosis.

Nuclear crowding (increased nuclear density). This change, associated with increased cellularity, is seen as a focus (Fig. 2A,B, 3A,B). This is easily recongizable by comparison with the non-tumorous hepatic parenchyma by an overall evaluation, and also by semiquantitative and quantitative analyses. This parameter exceeding twice that of normal tissue may be significant in this setting, and nuclear crowding is usually associated with an increased N/C ratio.

E. Cytoplasmic inclusions

The following inclusions in hepatocytes are valuable for identification as well as differential diagnosis.

Mallory body clustering. Mallory bodies containing hepatocytes adjacent to the portal tract or fibrous septa are seen in regenerative nodules and dysplastic nodules. This lesion without such polarity is focally seen in dysplastic nodules and low- grade HCC. In low-grade or classical HCC, Mallory bodies containing neoplastic hepatocytes without empty cytoplasms are clustered.

Pale bodies and hyaline droplets (globules). These are occasionally clustered in low-grade HCC or classical HCC, and are helpful for a differential diagnosis.

Bile plugs and/or feathery degeneration. In background livers without cholestasis, this can be a marker of dysplastic nodules, low-grade HCC or classical HCC. Feathery degeneration is rare in classical HCC.

F. Deposition of abnormal substances in the cytoplasm

The following abnormal substances are deposited in some hepatocellular nodules to a varied degree and extent. It is of interest that the nodules with increased depositions of fat, hemosiderin, and copper granules are easily detectable radiologically and then biopsied for a histological diagnosis.

Macrovesicular fat. This is accumulated in some

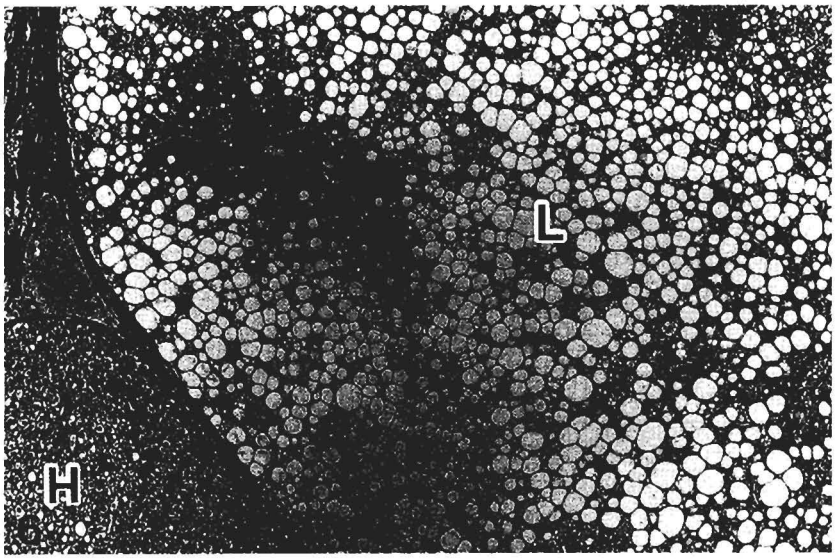

Fig. 6. This large regenerative nodule (surgically resected under a diagnosis of small hepatocellular carcinoma) shows marked marcrovesicular fatty change (L). Fatty change is rarely or only slightly seen in the surrounding liver (H). Chronic hepatis C. H&E, x 200

nodules. This lesion itself is not diagnostic and is seen in non-neoplastic (Fig. 6) as well as neoplastic nodules. Macrovesicular fat is, however, very useful for the identification of hepatocellular nodules in biopsied liver specimens.

Orcein-positive granules (copper granules). Orcein-positive granules are more or less suggestive of dysplastic nodules or low-grade HCC (Kitagawa et al., 1991). Some classical HCC also show concentrated copper granules in the cytoplasm.

Hemosiderin. Hemosiderin granules are abnormally deposited in some hepatocellular nodules to a varied degree and extent. This lesion itself is not diagnostic, but is useful for the identification of hepatocellular nodules. Foci of hemosiderin negative areas in dysplastic nodules are suggestive of the occurrence of malignant foci (low-grade HCC) (Nakanuma et al., 1993).

G. Increased cytoplasmic staining and clear cell change

These changes themselves are a good histological marker of the small nodule itself, but the information from these changes themselves is of limited use in differential diagnoses. These changes are seen in clustered hepatocytes or in all hepatocytes of a nodule.

Increased cytoplasmic staining. Eosinophilic, basophilic and ambophilic cytoplasmic staining is increased (Fig. 2B).

Clear cell change. The hepatocytes show clear cytoplasm and compress each other with indistinct sinusoids.

H. Dysplasia

Two types of liver cell dysplasia are known, and they each have their owen distict pathological significance. This term should not be confused with the term dysplastic nodule (see above).

Large cell dysplasia. Large cell dysplasia has been regarded as a risk factor predicting the occurrence of HCC (Anthony et al., 1973; Borzio et al., 1995). However, there is no evidence that a regenerative nodule with large cell dysplasia has udergone neoplastic transformation. This type of dysplasia may be more closely related to chronic hepatitis B viral infection (Akagi et al., 1984). Hytiroglou et al. (1995) recently proposed that liver cell dysplasia of the large cell type should not be used as a criterion for atypical cirrhotic nodules. The discrepancy of the meaning of this atypical lesion with respect to HCC may be due to the different criteria-used.

Small cell dysplasia. This is characterized by a cluster of small hepatocytes with an increased N/C ratio and also hyperchromatic nuclei. Cytoplasmic staining is also usually increased (Watanabe et al., 1983). There is an abrupt transition to the surrounding hepatocellular cords. Atrophic small hepatocytes with an increased N/C ratio and dilated sinusoids are not regarded as this lesion.

I. Sinusoidal changes

The following changes are some of the useful and reliable markers in differential diagnosis used at present.

Reduced or lost reticulin fibers. This is a very convenient and reproducible marker for distinguishing neoplastic nodules from dysplastic or regenerative nodules in routinely processed specimens (Nakanuma et al., 1993).

Capillarization around the hepatocellular cords. The capillarization of sinusoids is visualized by the immunostaining of CD34 (Tanigawa et al., 1997) and von Willebrand factor and the lectin immunohistochemistry of *Ulex europaeus* agglutinin-1. These endothelial markers are not expressed in normal hepatic sinusoids.

Scirrhous growth. This is characterized by a dense deposition of collagen fibers around the hepatocellular cords and is seen in some cases of low-grade HCC and classical HCC.

J. Expression of oncofetal markers

To date, no antigenic markers characterizing dysplastic nodules or low-grade HCC are known. Alpha-fetoprotein and mutant p53, a marker of classical HCC, are rarely detectable immunohistochemically in low-grade HCC and are not detected at all in dysplastic nodules or regenerative nodules (Tanaka et al., 1993). Enzyme deviation or alterations are not applicable to the differential diagnosis of HCC or its precursor (Schaff and Nagy, 1997).

Microscopic atypical foci in non-neoplastic hepatic parenchyma in chronic liver diseases

In chronic liver diseases, particularly liver cirrhosis, unusual or atypical changes resembling the histopathological changes seen in dysplastic nodules or low-grade HCC are also found in the non-neoplastic parenchyma with varied frequency: small foci of small cell dysplasia or large cell dysplasia, pseudogland formation, foci of hepatocytes showing cytoplasmic acidophilia or basophilia, clear cell change, peri-sinusoidal distribution of nuclei or nuclear irregularities, and thickened trabeculae. These changes are usually seen in some foci or about half of a regenerative nodule or hepatic lobule. Occasionally, such change(s) occupy a whole cirrhotic regenerative nodule of smaller size.

Similar changes of hepatocytes are also encountered in non-cirrhotic portal hypertension or within the

nodules of focal nodular hyperplasia, and also in primary biliary cirrhosis at non-cirrhotic stages (Nakanuma and Hirata, 1993). Thus, it seems reasonable to presume that a majority of these changes in regenerative nodules and hepatic lobules are reactive in character.

Ojanguren et al. (1997) recently surveyed such microscopic atypical lesions in 98 cases of chronic advanced liver diseases, and reported that cirrhotic nodules showing three or fewer changes were never associated with malignancy, whereas those exhibiting four or more alterations were often located in the vicinity of a tumor. Acinar structures, thickened cell trabeculae, a peripheral distribution of nuclei and nuclear irregularities seem to be the most specific indicators of proximity to HCC.

In any case, some of the above-mentioned unusual or atypical changes could be candidate markers of early neoplastic change. Ojanguren et al. (1997) suggested that such changes in cirrhotic nodules may also prove valuable in the selection of appropriate material for investigating early molecular events in hepatic carcinogenesis.

Differential diagnosis of small hepatocellular nodules by needle biopsies

Needle biopsies of the small hepatocellular nodules are frequently done to make a histological diagnosis. Because the histological changes applied to the differential diagnosis are limited in their occurrence and usually subtle or relatively strong or weak in individual livers, a simultaneous needle biopsy taken from the non-tumorous liver at a site remote from the nodule is strongly recommended. Our routine experience with this procedure is as follows.

First, the diagnosis of classical HCC is not difficult, and the differentiation from low-grade HCC or dysplastic nodules is also easily done with needle-biopsied specimens. Cellular and nuclear atypia and structural changes are useful in this differentiation.

Distinguishing dysplastic nodules from low-grade HCC

This histological differentiation is still a difficult task for histopathologists, particularly in the case of needle biopsy specimens. The histological findings or changes mentioned above should be searched for in the biopsy specimens and considered in the differentiation diagnosis. It should be mentioned that not all of these finding are detectable in a particular case. In other words, several histological markers or changes detectable in individual nodules should be analyzed carefully and comprehensively (Table 2). Particularly, the loss or reduction of reticulum fibers and capillarization, nuclear hyperchromasia and anisocytosis, large or clustered pseudogland, portal invasion and clear mosaic pattern at the periphery of the nodules, and iron-negative foci in siderotic dysplastic nodules, are frequently used and reproducible histological markers of low-grade HCC.

Small hypervascular nodules have recently become detectable in alcoholic cirrhosis under a radiological diagnosis of HCC: a majority of them are dysplasatic nodules or regenerative nodules.

Distinguishing dysplastic nodules from large regenerative nodules

In this distinction, clinical information regarding the exact hit of the biopsy needle on the aimed for nodule(s)

Table 2. A list of representative histopathological changes valuable for the differential diagnosis of small nodular lesions in chronic liver diseases.

HISTOPATHOLOGICAL FINDING	CLASSIFICATION OF SMALL HEPATOCELLULAR NODULES IN CHRONIC ADVANCED LIVER DISEASES				
	LRN without unusual lesions	LRN with unusual lesions	Dysplastic nodule	Low-grade HCC	Classical HCC
Polarity of hepatocytes	Preserved	Preserved	Preserved~disordered	Disordered~lost	Lost
Distribution of portal tracts	Regularly distributed	Regularly distributed	Irregularly distributed	Sparse or lost	Lost
Abnormal muscular vessels	Rare	Rare	Some	Some-increased	Increased
Portal tract invasion	-	-	- or vague	- or +	-
Venous invasion	-	-	-	±	++
Metastasis	-	-	-	-	++
Microacinus or pseudogland	-	Small and sparse	Small and focal	Large and clustered	Large and clustered
Nuclear changes	n.p.	n.p.	Nuclear crowding, hyperchromasia	Nuclear crowding, hyperchromasia, thick membrane	Aniso-nucleosis irregular contour
Mallory body clustering	Adjacent portal tracts	Adjacent to portal tracts	Irregular	Irregular	Irregular
Dense orcein(+) granules	-	-	Some cases	Some cases	Some cases
Lost or reduced reticulum fibers	-	-	-	-~extensive	-~extensive

LRN: large regenerative nodule; -: negative; ±: infrequent and focal; +: focal; ++: moderate to severe; n.p.: not particular.

and the histology of the needle biospy specimen from the nodule with that from the non-nodular liver should be carefully compared.

In dysplastic nodules, unusual lesions are seen in more than one regenerative nodule or hepatic lobule, while such changes are focal in regenerative nodules in the case of large regenerative nodules. In tiny fragmentous cores, it is impossible to distinguish dysplastic nodules from regenerative nodules, because regenerative nodules themselves contain hepatocellular foci showing unusual features seen in dysplastic nodules. Lastly, it should be remembered that the hepatic parenchyma or regenerative nodule near classical HCC frequently shows reactive atypical changes. At present, histopathologists can add this comment to a report of a needle liver biopsy to the clinician, noting that these atypical or unusual changes detected in cirrhotic regenerative nodules may indicate the probability that the patient has HCC elsewhere, particularly near the site aimed for by the biopsy.

Overview

In the light of recent recognition of two new small nodular lesions about 1 cm in diameter in chronic liver diseases, the tumor pathology of the liver and nosology of hepatocellular neoplasms should be reconsidered and revised. In addition, the detailed histological characteristics and definition of these two entities, their natural history, and their biological characters and corresponding genetic alterations, particularly the frequency of malignant progression, should be clarified by modern technology (Tsuda et al., 1988). It is also urgently necessary to clarify what percent of classical HCC detected clinically develop through this dysplastic nodule or low-grade HCC pathway.

Although various criteria and trials have been proposed for the identification of early neoplastic or morphological changes in the setting of both small HCC and dysplastic nodules, the precise histological changes preceding neoplastic lesions are still unclear.

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