Ductular Reaction Is Helpful in Defining Early Stromal Invasion, Small Hepatocellular Carcinomas, and Dysplastic Nodules

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BACKGROUND. Stromal invasion is 1 of the main features used to distinguish high-grade dysplastic nodules (DNs) from well-differentiated hepatocellular carcinomas (HCCs). The authors hypothesized that ductular reaction (DR) takes place around noninvasive hepatocellular nodules but not within the stroma contiguous to invasive HCC.

METHODS. DR/cytokeratin 7 (CK7)-positive patterns were evaluated in 105 resected small hepatic nodules according to the level of invasion. The nodules were classified histologically prior to immunostaining as noninvasive (large regenerative nodules, low-grade DNs, and high-grade DNs), minimally invasive (early HCCs with a vaguely nodular type), and overtly invasive (typical HCCs with a distinctly nodular type) in a review by expert pathologists, the current gold standard. Intranodular DR (inner DR) and DR around the nodule periphery (outer DR) were assessed separately on a semiquantitative scale from 0 to 4+.

RESULTS. DR was 3 or 4+ in the majority of noninvasive nodules (inner DR, 81%; outer DR, 91%), whereas DR was 0 or 1+ in overtly invasive HCCs (inner DR, 96%; outer DR, 81%). Minimally invasive HCCs showed an intermediate DR pattern (2 or 3+ inner DR, 75%; 2+ outer DR, 67%). DR characteristically was absent at the stromal-invasive, leading edge of tumor cells in both minimally invasive HCCs (focal loss of DR/CK7) and overtly invasive HCCs (diffuse loss of DR/CK7). The DR patterns in 41 needle-biopsy samples were similar to the patterns observed in resected nodules.

CONCLUSIONS. DR/CK7 immunostaining may help to identify small foci of invasion and to distinguish noninvasive, high-grade DNs from both minimally invasive and overtly invasive HCCs. ***Cancer* 2007;109:915–23.**

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The histologic details of early-stage human hepatocarcinogenesis have been well documented. Among the lesions that represent some of these early pathways, dysplastic nodules (DNs), especially

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high-grade DNs, currently are considered premalignant.[1–6] Although the first sign of malignancy in many epithelial malignancies is invasion through a preexisting basement membrane, this feature does not apply to hepatocellular lesions given the absence of a hepatocyte basement membrane. Instead, the histologic hallmark of transition to malignancy is now considered *stromal invasion*, that is, invasion into the portal tract or septal stroma within a hepatocellular (premalignant) nodule.

However, the identification of stromal invasion in patients with chronic liver disease, the clinical setting for most of these lesions, may be difficult in well-differentiated hepatocellular carcinomas (HCCs), including what Japanese researchers call *early HCCs*, because well-differentiated tumor cells show little or no cytologic atypia, and stromal invasion often is focal.[1,4,7,8] It is still more difficult when dealing with biopsied liver tissue, in which only limited portions of nodules are sampled. The differential diagnosis of such lesions would be hepatocytes surrounded by scar in the setting of chronic liver disease. Such small clusters of scar-enveloped hepatocytes have been referred to as hepatocyte *buds*.[9]

With 3-dimensional reconstructions, it has been demonstrated that these intraseptal hepatocyte *buds* are contiguous with ductular reactions (DRs) that are indicative of regeneration from intrabiliary progenitors.[10,11] Thus, stromal invasion by early, well-differentiated HCC, although it shares a superficial appearance with intraseptal hepatocyte buds, may differ, because the latter generally is accompanied by this histologic feature of progenitor cell activation and hepatobiliary differentiation (ie, DR). We hypothesize that such regenerative DR, highlighted by routine immunohistochemistry for biliary-type cytokeratin (CK7), is found around noninvasive hepatocellular nodules but not within the desmoplastic stroma contiguous to invasive HCC. The objectives of the current study were to test this hypothesis and to document whether the DR pattern, identified by CK7 immunostaining in surgical resections and biopsies of hepatocellular nodules, can help to highlight the presence of stromal invasion and, thereby, to identify well-differentiated HCC in nodular hepatocellular lesions from patients with chronic liver disease.

MATERIALS AND METHODS

Material and Pathologic Examination

Small hepatocellular nodules ($\leq$2 cm) that were diagnosed as large regenerative nodules (LRNs), DNs, or HCCs were selected from archival files of patients resection and the specimens were supported by the Liver Cancer Specimen Bank from the National Research Resource Bank Program of the Korea Science & Engineering Foundation in the Ministry of Science & Technology. Biopsied cases of small hepatic nodules also were collected.

The diagnosis of each hepatic nodule was made according to the criteria of the International Working Party as follows: 1) LRN, 2) low-grade DN, 3) high-grade DN, or 4) HCC.[12] LRN is a multiacinar, regenerative nodule that is distinctly larger than most cirrhotic nodules of the same liver, generally $\geq$0.5 cm in greatest dimension. DN is a nodular region of hepatocytes with dysplasia but without definite histologic criteria of malignancy; low-grade DN shows mild atypia, and high-grade DN shows at least moderate atypia. Low-grade DNs are differentiated from LRNs by features of large liver cell change, minimal nuclear abnormalities, and clone-like changes that are not detected in LRNs, such as diffuse hemosiderosis (in the absence of further distinguishing nodule-in-nodule lesions). High-grade DNs are characterized by 1) increased cellularity with a nuclear/cytoplasmic ratio 1.5 to 2 times more than that of adjacent cirrhotic nodules, 2) focal acinar arrangement, and 3) nodule-in-nodule lesions. Well-differentiated HCCs show increased cellularity and a nuclear/cytoplasmic ratio more than twice that of adjacent cirrhotic nodules and frequent acinar arrangement.[3,4,6,12,13]

Small HCCs ($\leq$2 cm) were subcategorized further into HCCs of vaguely nodular type and HCCs with distinctly nodular type based on macroscopic features.[1,4,13,14] The vaguely (indistinctly) nodular type retains the basic architecture of the background cirrhotic liver in varying degrees, and the cancer cells grow in a replacing trabecular pattern at the tumor/nontumor boundaries as though they were replacing the nonneoplastic liver cords. Thus, the tumor boundary is ill defined without tumor capsule. Histologically, most tumors of this type are well-differentiated HCCs with little cellular or architectural atypia and with a varying combination of the following features; 1) increased cellularity with increased nuclear/cytoplasmic ratio and 2) an irregular, thin, trabecular pattern 2 to 3 cells thick with frequent acinar arrangement and frequent fatty changes. Small HCC of the vaguely nodular type is considered as an early lesion and is designated as early HCC by Japanese researchers.[1,4,13,14] In contrast, small HCC of the distinctly nodular type is well demarcated, frequently encapsulated, and usually shows typical HCC histologic features. Therefore, biologically, it is considered a further step, compared with early HCC, on the route of cancer progression despite its small size.

The paraffin-embedded tissue from each nodule was sectioned serially and stained with hematoxylin

and eosin (H&E). The categorical diagnostic assignments for each of the hepatic nodules in this study were arrived at by consensus between 2 or 3 of the participating pathologists and discussions over a multiheaded microscope. The diagnoses were based on H&E features and, when necessary, by additional CD34 immunostaining to highlight sinusoidal capillarization, which generally was focal in high-grade DNs and diffuse in well-differentiated HCCs.[15–17]

Evaluation of DR

Immunohistochemical staining for CK7 (monoclonal; DAKO, Carpenteria, CA; dilution, 1:100) was performed by using an LSAB kit (DAKO) as described previously.[18] CK7-immunoreactive DRs were analyzed at the epithelial-stromal boundaries within and at the outer edge of each nodule. The intranodular (inner) DR was semiquantified as follows: 0 = none, 1 = <10%, 2 = 10% to 25%, 3 = 26% to 50%, and 4 = >50% of the epithelial-stromal interface involved by DR/CK7. The peripheral (outer) DR between the lesional nodule and the surrounding liver tissue was scored similarly: 0 = none, 1 = <10%, 2 = 10% to 25%, 3 = 26% to 50%, and 4 = >50% of the nodular circumference involved by DR/CK7. The differences in the extent of DR among the categories of hepatocellular nodules were evaluated statistically by using the Fisher exact test.

RESULTS

DR in Resected Hepatic Nodules

The pathologic diagnoses of the 105 resected hepatic nodules that were included in this study were LRN (n = 10 nodules), low-grade DN (n = 28 nodules), high-grade DN (n = 29 nodules), small HCC of the vaguely nodular type (n = 12 nodules), and small HCC of the distinctly nodular type (n = 26 nodules). The relevant clinicopathologic information is summarized in Table 1.

Morphologically identifiable stromal invasion was not observed in nonmalignant hepatocellular nodules (LRN, low-grade DN, and high-grade DN) but only in histologically defined HCC. In well-differentiated HCC of the vaguely nodular type, stromal invasion was focal and subtle. It also may have been present in some entrapped intralesional portal tracts, suggesting a minimally invasive type of HCC. In HCC of the distinctly nodular type, stromal invasion was more frequent and obvious, indicating a more overtly invasive phenotype of HCC.

DRs were positive uniformly and strongly for CK7. Intraseptal hepatocytes and noninvasive hepatocyte-stromal boundaries revealed CK7-positive DR (Fig. 1). In contrast, HCC cells that invaded entrapped intralesional portal tracts and fibrous stroma within HCC showed no CK7-positive DR (Fig. 2).

Inner DR results are summarized in Table 2. Inner DR was florid (3+ or 4+) in 54 of 67 of noninvasive type nodules (81%), including LRNs, low-grade DNs, and high-grade DNs. In contrast, overtly invasive HCCs of the distinctly nodular type showed little or negative inner DR (0 or 1+) in 25 of 26 samples (96%). Therefore, the inner DR pattern differed significantly between noninvasive hepatic nodules and invasive HCCs ($P < .001$). In well-differentiated HCCs of the vaguely nodular type that showed a doubtful, minimally invasive phenotype on H&E examination, inner DR was 2 or 3+ in 9 of 12 samples (75%). The invasive foci were distributed focally and, overall, a retained CK7-positive DR was observed at the epithelial-stromal interface. However, the extent of DR in vaguely nodular DN was significantly higher than that detectable in the overtly invasive HCCs (small HCCs of the distinctly nodular type), which showed little or no DR in most samples (25 of 26 nodules; 96%; $P < .001$). The inner DR pattern of minimally invasive HCCs overlapped to some extent with the pattern observed in noninvasive type nodules, which showed 2 or 3+ DR in the majority of samples (44 of 67 nodules; 66%). The focal but unequivocal loss of CK7-positive DR at the leading edge of HCC with stromal invasion helped to distinguish minimally invasive HCCs of the vaguely nodular type from all nonmalignant hepatocellular nodules (Fig. 3).

Outer DR findings are summarized in Table 3. The degree of outer DR differed significantly ($P < .001$) between nodules according to the presence or absence of stromal invasion. All noninvasive nodules, including LRNs, low-grade DNs, and high-grade DNs, showed florid outer DR; 4+ outer DR was observed in 10 of 10 LRNS (100%), in 15 of 28 low-grade DNs (54%), and in 16 of 29 high-grade DNs (55%) (Fig. 1). In contrast, outer DR was absent or scant (0 or 1+) in 21 of 26 HCCs of the distinctly nodular type (81%), in 13 of 26 HCCs (50%) showed no outer DR, and 8 of 26 HCCs (31%) showed 1+ outer DR (Fig. 2); whereas 5 of 15 moderately differentiated HCCs of the distinctly nodular type (33%) showed 2+ or 3+ outer DR. This may be considered a noninvasive region of HCC with invasion elsewhere or, alternatively, non-neoplastic residual-entrapped portal tracts at the edge of invasive tumor. In these tumors, the identification of stromal invasion was not problematic because of more obvious histologic and cytologic atypia. Well-differentiated HCCs of the vaguely nodular type with minimal invasion showed 2+ outer DR in most samples (8 of 12 nodules; 67%); however, in 1 nodule (8%) that showed focal stromal invasion within the nodule, 4+ florid outer DR was observed (Fig. 3).

TABLE 1
Clinicopathologic Data on Resected Hepatic Nodules

Lesion	Mean age (Range), y	Sex (M:W)	Mean lesion size (Range), cm	Etiology, No. of patients				No. with cirrhosis [%]*
				HBV	HCV	Alc	Biliary	
LRN, n = 10	54.8 (43–62)	8:2	0.6 (0.5–1.3)	9	0	1	0	10 [100]
LGDN, n = 28	55.2 (44–72)	20:8	1.0 (0.5–1.5)	25	2	0	1	26 [93]
HGDN, n = 29	55.5 (44–68)	20:9	1.2 (0.6–1.9)	25	4	0	0	29 [100]
Small HCC, vaguely nodular type, WD, n = 12	63.4 (51–80)	7:5	1.2 (0.7–1.8)	6	5	0	1	12 [100]
Small HCC, distinctly nodular type, n = 26	52.6 (43–71)	18:8	1.5 (0.7–2.1)	15	10	1	0	24 [92]
Small HCC, distinctly nodular type, WD, n = 11	56.4 (43–71)	7:4	1.4 (0.7–2.1)	5	6	0	0	10 [91]
Small HCC, distinctly nodular type, MD, n = 15	55.1(43–64)	11:4	1.5 (1.0–2.0)	10	4	1	0	14 [93]

M:W indicates the ratio of men to women; HBV, hepatitis B virus; HCV, hepatitis C virus; Alc, alcoholic; LRN, large regenerative nodule; LGDN, low-grade dysplastic nodule; HGDN, high-grade dysplastic nodule; HCC, hepatocellular carcinoma; WD, well differentiated; MD, moderately differentiated.
* Cirrhosis in the background liver.

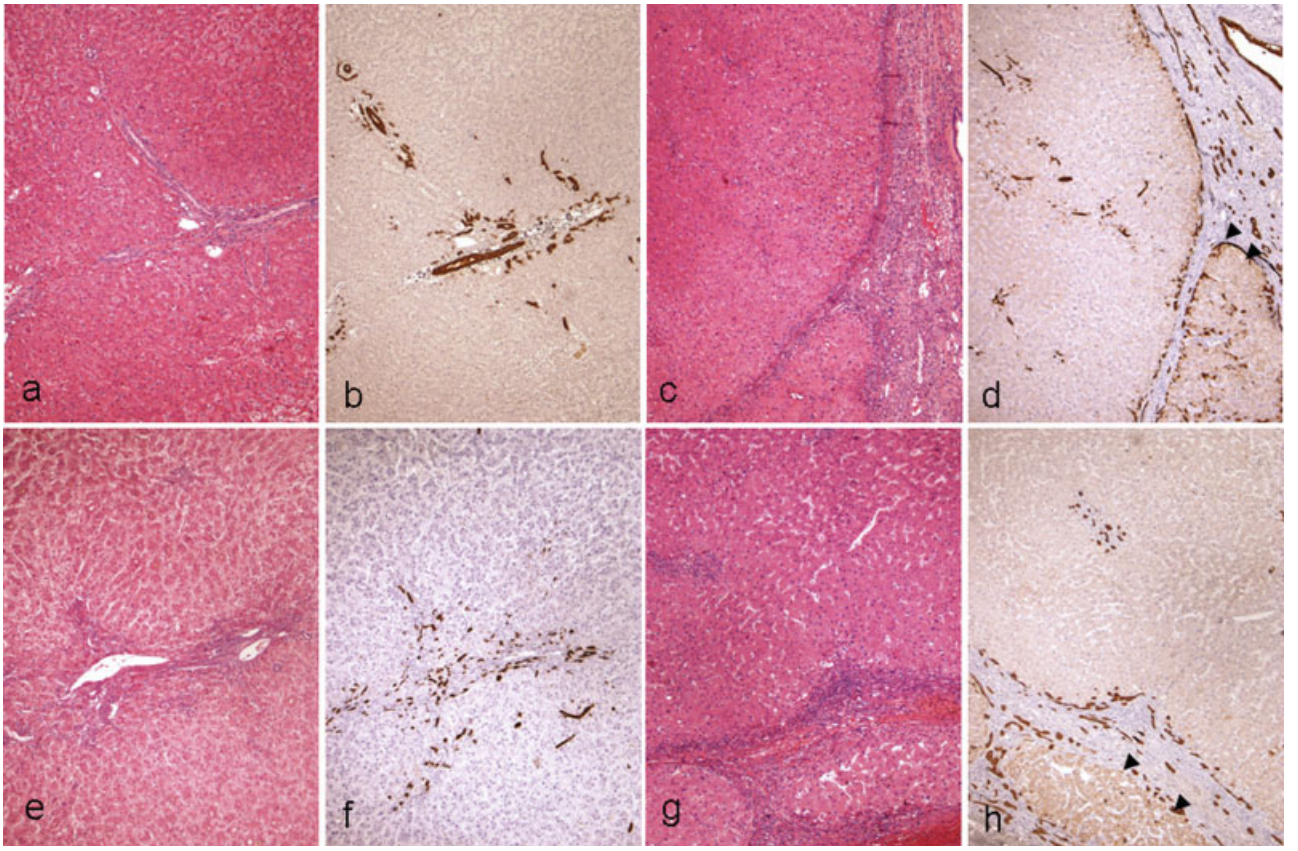

FIGURE 1. Ductular reaction (DR) patterns of dysplastic nodules. Intraseptal hepatocytes at the epithelial-stromal border showing cytokeratin 7 (CK7)-positive DR, indicating a noninvasive pattern. (a-d) A low-grade dysplastic nodule with inner DR within the nodule and with outer DR between the nodule and non-nodular areas. (e-h) A high-grade dysplastic nodule with inner DR within the nodule (e,f) and with outer DR between the nodule and non-nodular areas (g,h). DR is florid around cirrhotic nodules (arrows in d,h) (hematoxylin and eosin, immunohistochemical stain for CK7).

DR in Biopsied Hepatic Nodules

The morphologic diagnoses of the 41 needle-biopsied samples of small hepatocellular nodules that we studied were LRNs (n = 7 nodules), low-grade DNs (n = 4 nodules), high-grade DNs (n = 5 nodules), and well-differentiated HCCs (n = 21 nodules). There were 4 borderline results in which the distinction between high-grade DN and HCC was uncertain with

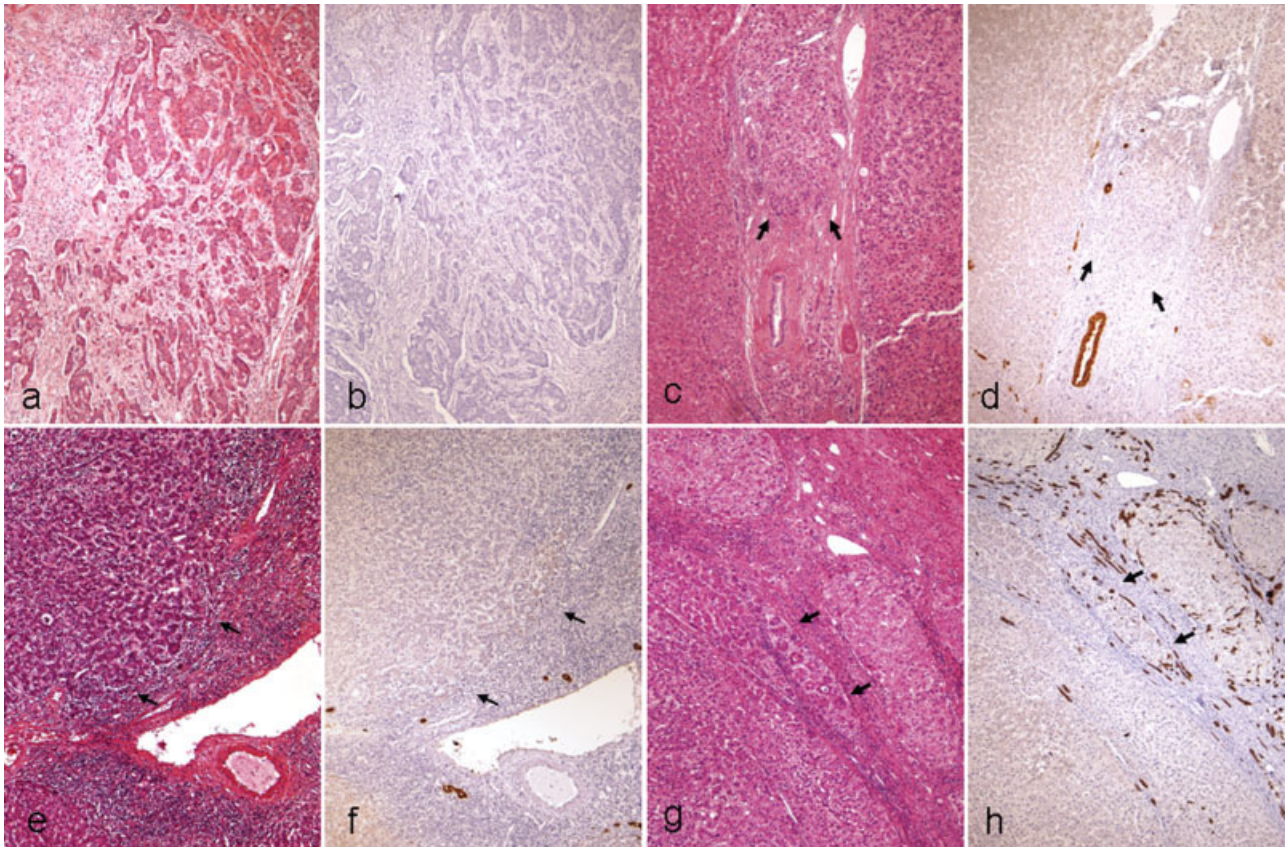

FIGURE 2. Ductular reaction (DR) patterns of overtly invasive hepatocellular carcinoma (HCC). (a-d) Inner DR is absent in areas of stromal invasion (a,b) and portal tract invasion (c,d, arrows). The bile duct is positive for cytokeratin-7 (CK7). (e,f) The outer border of the lesion (arrows) shows no CK7-positive DR, indicating an overtly invasive HCC phenotype. (g,h) Rare, overtly invasive type HCC shows focal (2+) outer DR (arrows), which may be a noninvasive component of the invasive HCC or residual-entrapped portal tracts within the invasive tumor (hematoxylin and eosin, immunohistochemical stain for CK7).

TABLE 2
Inner Ductular Reaction at Intralesional Epithelial-Stromal Interface

	Degree of inner ductular reaction				
Lesion	**0**	**1+**	**2+**	**3+**	**4+**
LRN, n = 10	0	0	1	2	7
LGDN, n = 28	0	0	4	15	9
HGDN, n = 29	0	2	6	16	5
Small HCC, vaguely nodular type, WD, n = 12	0	3	5	4	0
Small HCC, distinctly nodular type, n = 26	15	10	1	0	0
Small HCC, distinctly nodular type, WD, n = 11	8	3	0	0	0
Small HCC, distinctly nodular type, MD, n = 15	7	7	1	0	0

LRN indicates large regenerative nodule; LGDN, low-grade dysplastic nodule; HGDN, high-grade dysplastic nodule; HCC, hepatocellular carcinoma; WD, well differentiated; MD, moderately differentiated.

* The degree of inner ductular reaction was scored as the percentage of the epithelial-stromal interface involved by cytokeratin 7/ductular reaction as follows: 0 = none, 1 = <10%, 2 = 10%–25%, 3 = 26%–50%, and 4 = >50%.

the needle-core sampling. The clinicopathologic features are summarized in Table 4.

It was not possible to assess inner DR and outer DR separately in the biopsied nodules because of the lack of a clear nodular topography in most of the small liver fragments. DR generally was semiquantified at the epithelial-stromal interface, and the findings are summarized in Table 5. The degree of DR differed significantly between clearly invasive and noninvasive lesions ($P < .001$).

All noninvasive hepatocellular nodules (LRNs, low-grade DNs, and high-grade DNs) showed florid DR at the epithelial-stromal boundaries (4+ DR in 7 of 16 nodules [44%] and 3+ in 6 of 16 nodules [38%]), and there were no DR-negative or 1+ DR nodules. Sixteen of 21 HCCs (76%) showed no DR, in keeping with an overtly invasive phenotype; whereas 5 of 21 HCCs (24%) showed residual DR and had morphology consistent with a minimally invasive

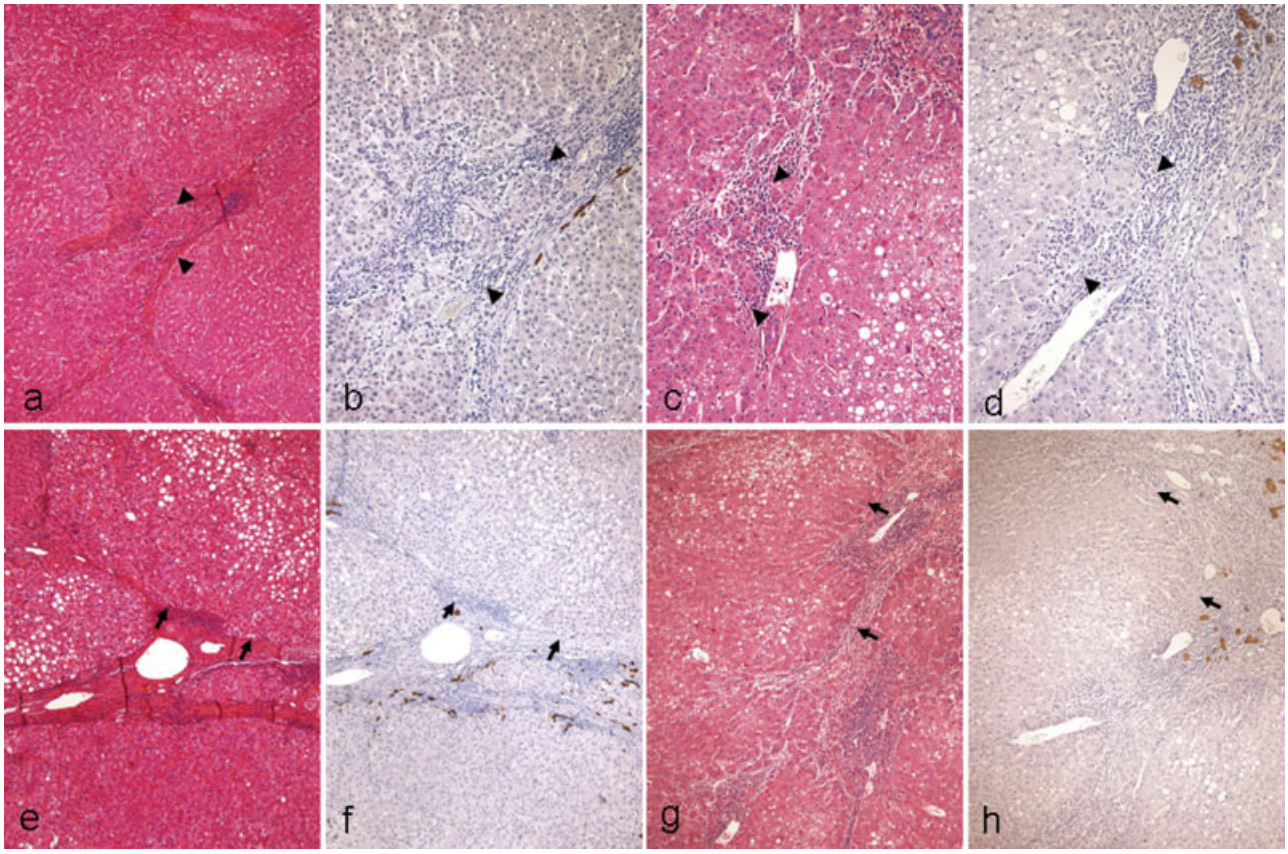

FIGURE 3. Ductular reaction (DR) patterns in minimally invasive hepatocellular carcinoma (HCC) of the vaguely nodular type (early HCC). (a-d) The intranodular, invasive, leading edge of the tumor is indicated by arrowheads. Inner cytokeratin 7 (CK7)-positive DR is not present apart from some residual CK7-positive DR in the entrapped portal tract. (e-h) Outer CK7/DR is scanty (arrows) between the lesion and the adjacent liver tissue in contrast to CK7-positive DR of the cirrhotic nodules around the lesion (hematoxylin and eosin, immunohistochemical stain for CK7).

TABLE 3
Outer Ductular Reaction at the Fibrous Border between Nodular Lesion and Nonlesional Liver Tissue

	Degree of outer ductular reaction*				
Lesion	**0**	**1+**	**2+**	**3+**	**4+**
LRN, n = 10	0	0	0	0	10
LGDN, n = 28	0	0	4	9	15
HGDN, n = 29	0	1	1	11	16
Small HCC, vaguely nodular type, WD, n = 12	0	1	8	2	1†
Small HCC, distinctly nodular type, n = 26	13	8	2	3	0
Small HCC, distinctly nodular type, WD, n = 11	8	3	0	0	0
Small HCC, distinctly nodular type, MD, n = 15	5	5	2	3	0

LRN indicates large regenerative nodule; LGDN, low-grade dysplastic nodule; HGDN, high-grade dysplastic nodule; HCC, hepatocellular carcinoma; WD, well differentiated; MD, moderately differentiated.

* The degree of outer ductular reaction was scored as the percentage of nodular circumference involved by cytokeratin 7/ductular reaction as follows: 0 = none, 1 = <10%, 2 = 10%–25%, 3 = 26%–50%, and 4 = >50%.

† This sample showed focal stromal invasion (negative ductular reaction area) within the nodule.

phenotype of HCC, with partly invasive (DR-negative) and partly noninvasive (DR-positive) areas in the same biopsy (Fig. 4). In 2 of 4 borderline nodules (DN/HCC), DR-negative/DR1+ areas were observed focally. The other 2 borderline lesions showed DR-positive areas (DR2+ or 3+).

DISCUSSION

Stromal invasion has been proposed as an important diagnostic feature of HCC, although its appearance, particularly for the inexperienced liver pathologist without extensive exposure to resected hepatocellular nodules or explanted cirrhotic livers, can be similar to regenerative intraseptal hepatocyte *buds*. It was demonstrated previously that these non-neoplastic, intraseptal hepatocytes are associated with DR in chronic liver diseases.[10] Because the most proximal branches of the biliary tree (ie, the canals of Hering and ductules) comprise or at least harbor facultative hepatic stem cells,[10,11] using the terminology of Wanless et al.,[9] these intraseptal hepato-

TABLE 4
Clinicopathologic Data on Hepatic Nodular Lesions With Biopsy Samples

Lesion	Mean age (Range), years	Sex (M:W)	Etiology					
			HBV	HCV	Alcoholic	Cryptogenetic	Dysmetabolic	Unknown
LRN (n = 7)	65.7 (60–71)	6:1	0	6	0	0	0	1
LGDN (n = 4)	65.5 (58–77)	3:1	1	1	2	0	0	0
HGDN (n = 5)	59.6 (41–72)	4:1	0	2	1	0	0	2
Borderline lesions (n = 4)	60 (51–65)	3:1	2	1	0	1	0	0
HCC (n = 21)	78 (58–79)	17:4	5	7	0	0	4	5

M:W indicates the ratio of men to women; HBV, hepatitis B virus; HCV, hepatitis C virus; LRN, large regenerative nodule; LGDN, low-grade dysplastic nodule; HGDN, high-grade dysplastic nodule; HCC, hepatocellular carcinoma.

TABLE 5
Ductular Reaction at the Fibrous Border Between Nodule and Nonnodule in Biopsy Samples

Lesion	Degree of ductular reaction*				
	0	1+	2+	3+	4+
LRN (n = 7)	0	0	2	2	3
LGDN (n = 4)	0	0	0	2	2
HGDN (n = 5)	0	0	1	2	2
Borderline lesions (n = 4)	0	2	1	1	0
HCC (n = 21)	16	4	1	0	0

LRN indicates large regenerative nodule; LGDN, low-grade dysplastic nodule; HGDN, high-grade dysplastic nodule; HCC, hepatocellular carcinoma.

* The degree of ductular reaction was scored as the percentage of epithelial-stromal interface involved by cytokeratin 7/ductular reaction as follows: 0 = none, 1 = <10%, 2 = 10%–25%, 3 = 26%–50%, and 4 = >50%.

cytes probably represent *buds* of newly formed hepatocytes arising from branches of the biliary tree.

Based on this understanding, we hypothesized that, although DR is observed around noninvasive (benign) hepatocellular nodules, it would not be present within the preexisting portal/septal stroma or the reactive desmoplastic stroma invaded by HCC. The results from the current study confirmed that noninvasive hepatocellular-stromal boundaries were associated with CK7-positive DRs, whereas the areas of morphologically identified stromal invasion did not show CK7-positive DR. Inner DR and outer DR were obvious and florid (4+) in the majority of noninvasive nodules, such as LRNs, low-grade DNs, and high-grade DNs. In contrast, inner DR and outer DR were scant or absent (1+ or 0) in most HCCs of the distinctly nodular type, showing an overtly invasive phenotype. With regard to well-differentiated HCCs of the so-called vaguely nodular type (early HCC), which showed the minimally invasive phenotype, the degrees of inner DR and outer DR were intermediate between those observed in noninvasive hepatic nodules and overtly invasive HCCs. CK7 DR usually was absent in the small foci of invasive tumor in minimally invasive HCCs, which distinguished it from both dysplastic nodules (without focal DR loss) and overtly invasive HCCs (with diffuse DR loss).

Most malignant and nonmalignant nodules contained some residual DR. DR scores of 2+ and 3+ were most prevalent in noninvasive nodules and also were observed in minimally invasive HCCs, in which DR was observed around potentially premalignant regions. Focal DR also could be seen rarely in overtly invasive HCC, which emphasizes the concept that the DR-negative focus is the salient feature that supports an interpretation of stromal invasion, rather than reliance on the DR semiquantification that was used in the current study for the purpose of data analysis.

Small HCCs ($\leq$2cm) can be subclassified into HCCs of a vaguely nodular type (so called early HCCs) and HCCs of a distinctly nodular type. Tumor cell invasion into the portal vein and minute intrahepatic metastases in the proximity of the tumor are observed in 27% and 10% of distinctly nodular HCCs, respectively, but are not observed in vaguely nodular HCCs.[1,4] Therefore, vaguely nodular HCCs likely correspond to carcinoma in situ and minimally invasive (or microinvasive) carcinoma of other organs; in contrast, distinctly nodular HCCs are biologically more advanced HCCs despite their small size. The identification of early HCCs is important for more effective radical surgical (or ablation) therapy.

Stromal invasion, which is an objective criterion of carcinoma, can be identified with relative ease in most HCCs of the distinctly nodular type (typical HCCs). However, stromal invasion is obscure in well-differentiated HCCs of the vaguely nodular type (early HCCs), because any such invasion is focal, often minimal, and has little cytologic or structural

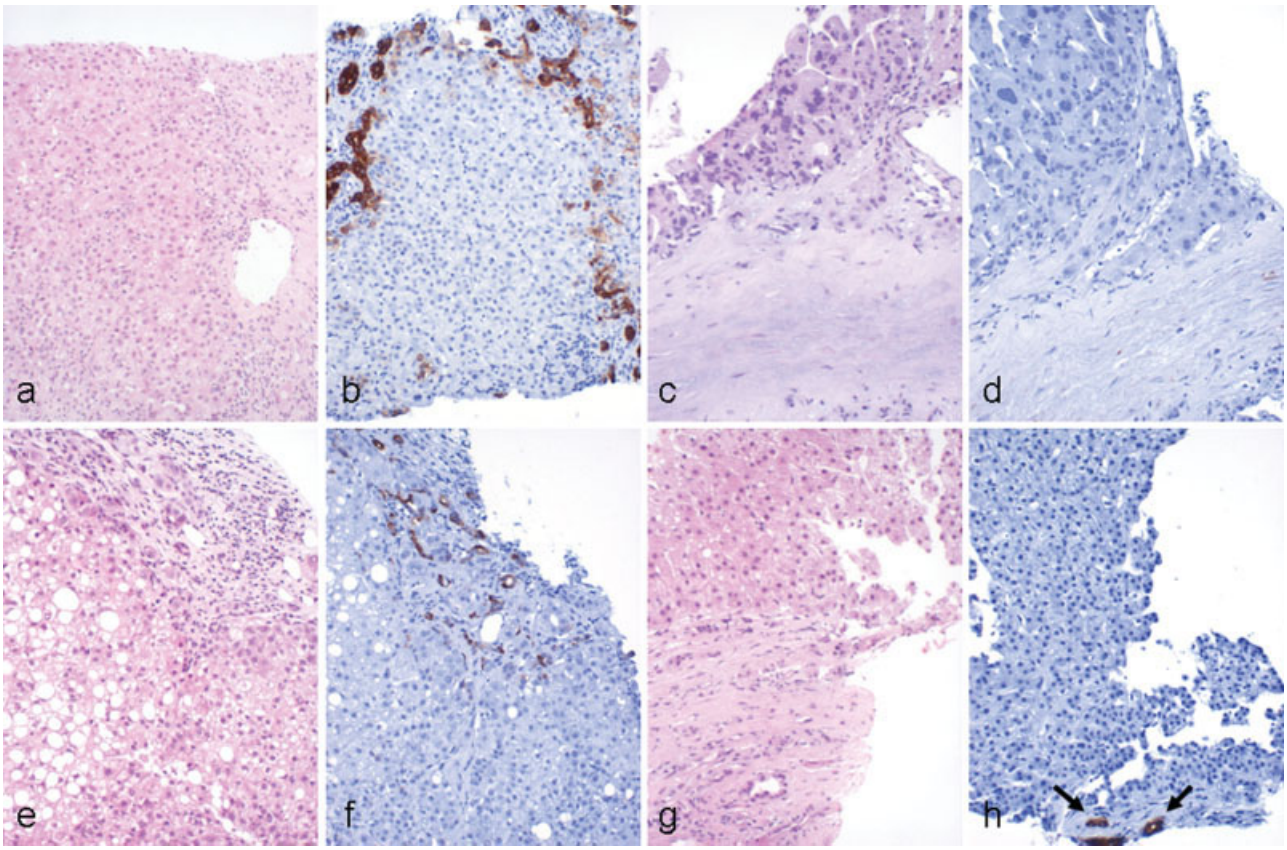

FIGURE 4. Ductular reaction (DR) patterns in biopsy samples from hepatocellular nodular lesions. (a,b) A high-grade dysplastic nodule with florid peripheral DR indicates a noninvasive (DR-positive) lesion. (c,d) Hepatocellular carcinoma shows no DR at the epithelial-stromal interface, indicating an invasive (DR-negative) phenotype. (e-h) Biopsied hepatic nodule with morphologic atypia and both DR-positive areas (e,f) and DR-negative areas (g,h) at the epithelial-stromal interface in the same biopsy, suggesting a minimally invasive phenotype (mixed DR-positive/DR-negative). Cytokeratin 7 (CK7)-positive immunoreactive structures (arrows in h) are preexisting bile ducts entrapped within the lesion (hematoxylin and eosin, immunohistochemical stain for CK7).

atypia. The identification of stromal invasion is particularly difficult in liver biopsy samples.[7,8] Although the pathologists who contributed to this study are recognized experts in this diagnostic area, and, indeed, such expert review is considered the gold standard of diagnosis, this standard is of limited practicality, because the majority of liver pathologists lack the opportunity to examine resected nodules or cirrhotic explants consistently for the development of such expertise. Therefore, the recognition of subtle lesions of stromal invasion can be difficult. Our finding of the absence of CK7-positive DRs in foci of expert-recognized stromal invasion suggests that immunostaining for these structures may be a useful adjunct for this diagnostic dilemma and may help to sensitize pathologists to the appearance of the histologic lesion. Recently, several novel immunohistochemical and molecular markers of hepatocellular malignancy have been proposed, including glypican 3,[19] heat-shock protein 70,[20] glutamine synthetase,[21] and human telomerase reverse transcriptase.[22] Because CK immunostaining cannot address, per se, all of the problematic cases, we suggest that this antibody probably is most informative if it is used within a panel of markers of hepatocellular malignancy.

In conclusion, the current results demonstrate that expert-identified stromal invasion, even of well-differentiated HCC, is devoid of CK7-positive DR. DR remains a sign of newly regenerating hepatocytes in chronic liver disease and, thus, does not also develop either from biliary metaplasia of malignant hepatocytes or from the outgrowth of biliary cells to contact these invading cancer cells. The finding that many of these well-differentiated HCCs are not overtly cholestatic suggests that bile excretion is maintained through canalicular links between malignant and neighboring benign hepatocytes, either by loss of the bile-producing metabolic pathways in the malignant cells or by retrograde, basilar excretion of bile in malignant hepatocytes.

We also believe that the absence of CK7-positive DR is a useful marker for characterizing areas of stromal invasion in small HCC and, thus, for distinguishing noninvasive hepatocellular lesions (DNs), minimally invasive HCC of the vaguely nodular type (early HCCs), and overtly invasive HCCs of the nodular type (typical/classic HCC with stromal invasion). In the setting of cirrhosis, these hepatocellular nodules pose an increasingly frequent differential diagnostic problem, although few pathologists have had consistent experience with them. Thus, we suggest that the assessment of CK7-positive DRs may improve diagnostic confidence in both needle-biopsy specimens and resection specimens for nonexpert pathologists who are faced with the spectrum of lesions that occurs in small hepatocellular nodules.

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