Hepatocellular Carcinoma Precursor Lesions

6

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Abstract

There are several precursor lesions to hepatocellular carcinoma, including small cell change, large cell change, and dysplastic nodules. Small cell change and large cell change are microscopic findings typically found in cirrhotic livers, while dysplastic nodules are commonly identified either by imaging or gross examination. The histological features and a diagnostic approach to each of these entities is discussed and illustrated.

Keywords

Small cell change • Large cell change • Macroregenerative nodule • Low-grade dysplastic nodule • High-grade dysplastic nodule • Hepatocellular carcinoma • Glypican-3 • Reticulin • CD34 • Glutamine synthetase • Nodule-in-nodule

6.1 Small Cell Change and Large Cell Change

6.1.1 Definition

Dysplastic hepatocyte foci are microscopic clusters of hepatocytes showing cytological characteristics that are different from the surrounding hepatocytes. In 1995, the International Working Party added criteria that dysplastic foci should measure less than 1 mm [1]. While any size criteria is necessarily arbitrary, this criteria does emphasize that these lesions are small and visible only on microscopy. Dysplastic foci are further subdivided by cytological criteria into large cell change and small cell change [2]. Large cell change and small cell change may be seen as independent findings, but also can be found within dysplastic nodules.

Large cell change and small cell change are strongly associated with chronic injury and are almost always found in the setting of cirrhosis, where the underlying liver disease results in DNA changes that can manifest as small or large cell change. Both large cell change and small

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cell change have been associated with subsequent development of hepatocellular carcinoma [2–4]. Large cell change, small cell change, and dysplastic nodules are commonly found in explanted livers, but the clinical significance in this setting is diminished, because the liver is fully resected, though this is an important source of material for studies to characterize the morphology and molecular findings.

6.1.2 Clinical Findings

Large cell change and small cell change are not visible radiographically and there are no reported serological or laboratory findings. However, they can be identified as a component of dysplastic nodules or macroregenerative nodules, which may be visible by imaging studies. Large cell change is commonly seen in chronic hepatitis B with advanced fibrosis, but can be seen in cirrhosis from any cause, and less commonly in non-cirrhotic livers with chronic liver disease. Several studies have found an association with large cell change and older age at biopsy or resection [5–7].

Molecular studies indicate small cell change is a direct precursor lesion to hepatocellular carcinoma [3]. Large cell change seen on liver biopsy is also associated with an increased risk for subsequent hepatocellular carcinoma [8–11]. However, the molecular data and clinical follow-up data is mixed on whether all cases of large cell change are precancerous, and in some cases large cell change may be a marker of increased risk for hepatocellular carcinoma, without necessarily being a direct precursor [10]. For example, in cholestatic liver disease, large cell change appears more likely to be reactive. In an excellent review article on large cell change, the authors point out that the absence of large cell change on liver biopsy has a strong negative predictive value for developing hepatocellular carcinoma, indicating that individuals without large cell change on biopsy have a lower probability of hepatocellular carcinoma development within the next 3–5 years [12].

6.1.3 Microscopic Findings

Large cell change is characterized by clusters of hepatocytes that show a variety of cytological changes including multi-nucleation, hyperchromasia, irregular nuclear contours, nuclear enlargement, cytoplasmic expansion, and nuclear pleomorphism (Fig. 6.1) [5]. In contrast to small cell change, the overall nuclear-to-cytoplasmic ratio is preserved in large cell change. Large cell change is usually easy to recognize, particularly in comparison to adjacent normal hepatocytes (Fig. 6.2). Large cell change is commonly persistent over time and is frequently present in follow-up biopsy specimens.

Hepatocytes with small cell change show slight nuclear enlargement and a decrease in cytoplasm, resulting in an increased nuclear-to-cytoplasmic ratio (Fig. 6.3). This in turn leads to a marked increase in nuclear density, compared to the adjacent liver (Fig. 6.4) [2]. Small cell change also shows nuclear and cytoplasmic basophilia, giving the lesion a “darker” appearance when compared to adjacent normal hepatocytes.

6.1.4 Immunohistochemical Findings

There are no useful diagnostic immunostains to identify large cell and small cell change, and the diagnosis is made on H&E stain.

6.1.5 Molecular Findings

Large cell change shows variable molecular findings, with evidence that in some cases the changes are reactive and perhaps senescent, while in other cases the changes are likely preneoplastic [12–14]. Small cell change is associated with chromosomal abnormalities, including telomere shorting, tumor suppressor gene inactivation (such as the p21 check point), and a higher proliferation rate [3, 15]. These molecular and chromosomal changes support a model in which small cell change is a direct precursor to hepatocellular carcinoma.

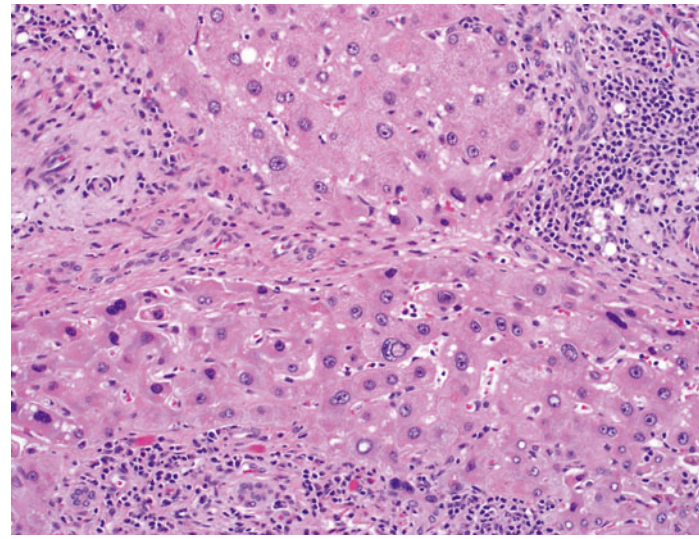

Fig. 6.1 Large cell change. Hepatocytes show nuclear and cytoplasmic enlargement with preserved nuclear-to-cytoplasmic ratios. Additional cytological changes include multinucleation, hyperchromasia, irregular nuclear contours, nuclear inclusions, and nuclear pleomorphism

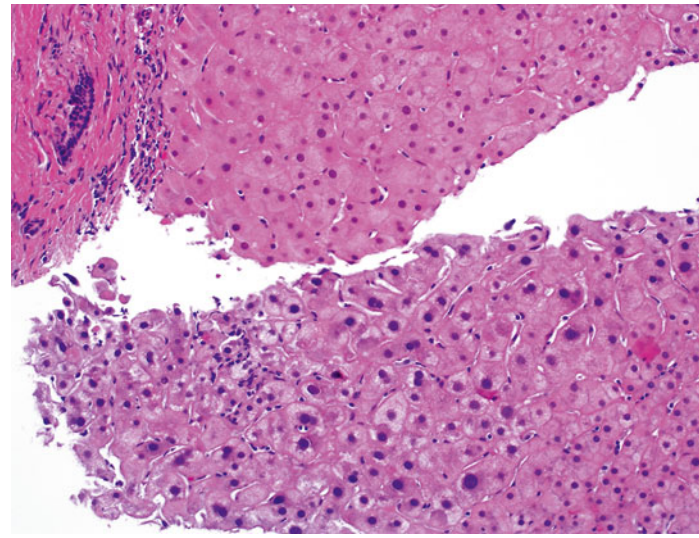

Fig. 6.2 Large cell change in comparison to normal hepatocytes. Note the enlarged and hyperchromatic cells (*bottom*) with preserved nuclear-to-cytoplasmic ratios. This focus of large cell change was identified in the adjacent benign liver from a biopsy that showed hepatocellular carcinoma elsewhere

6.1.6 Differential Diagnosis

One mimic of large cell change is regenerative and reactive hepatocytes in the setting of lobular injury and repair. However, the distinction is usually straightforward if attention is paid to the nuclear-to-cytoplasmic ratio and nuclear atypia, including multinucleation.

Atrophic hepatocytes can mimic small cell change, showing an increase in nuclear-to-cytoplasmic ratio and mild increased nuclear density. One example is nodular regenerative hyperplasia, where atrophic hepatocytes share some cytological similarities to small cell change. However, small cell change in most cases can be readily distinguished from atrophic hepatocytes by the presence of nuclear atypia and careful examination of the remainder of the biopsy findings. Keep in mind that large cell change and small cell change are most often seen in the setting of advanced liver fibrosis.

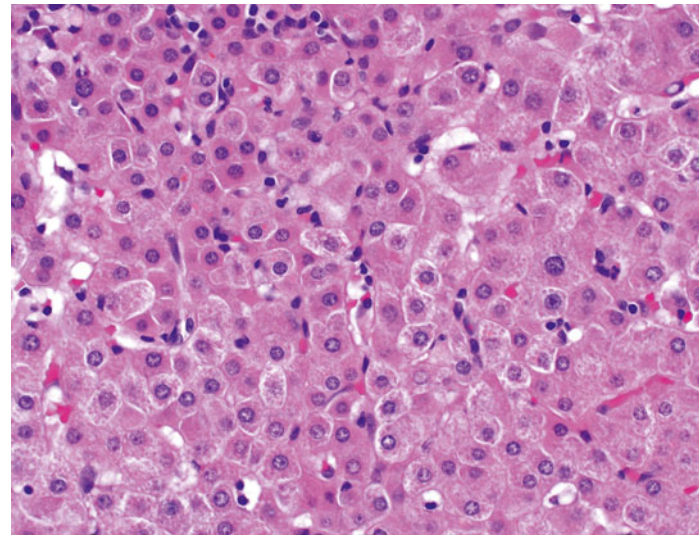

Fig. 6.3 Small cell change. The cells show mild nuclear enlargement, combined with a reduction in cytoplasm, resulting in increased nuclear-to-cytoplasmic ratios. The nuclei and cytoplasm show increased basophilia

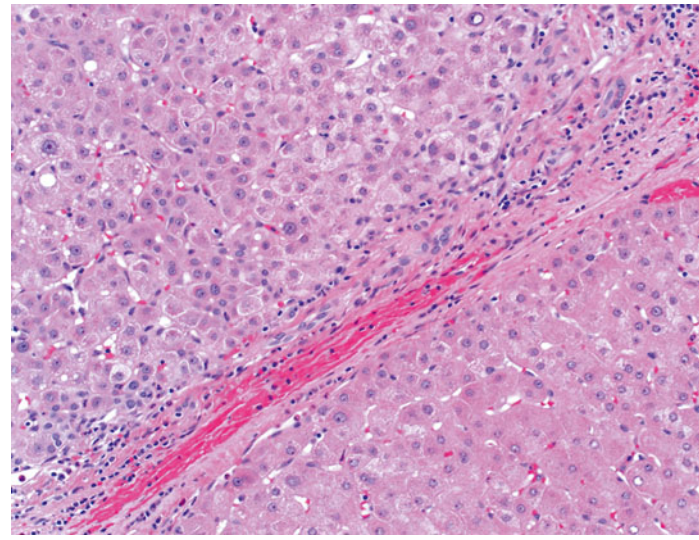

Fig. 6.4 Small cell change. A focus of small cell change (*top-left*) adjacent to a nodule of cirrhosis (*bottom-right*). Note the increased nuclear-to-cytoplasmic ratio and relative increased basophilia

The diagnosis of small cell change and large cell change will most often be made in the setting of non-targeted biopsies of livers with chronic disease, such as chronic viral hepatitis. These dysplastic foci are microscopic abnormalities, usually measuring less than 1 mm in diameter. Larger lesions, or lesions seen by imaging, will fall into the categories of macroregenerative nodules, dysplastic nodules, or hepatocellular carcinoma.

6.2 Macroregenerative and Dysplastic Nodules

6.2.1 Definition/Nomenclature

Dysplastic nodules are defined grossly as nodular lesions that are distinctly different from adjacent cirrhotic nodules with respect to size, color, texture, and/or degree of bulging from the cut surface. Although the gross characteristic help in

directing sampling of resected specimens, the diagnosis of these lesions requires histologic examination.

There is a continuous morphological progression from macroregenerative nodules to dysplastic nodules with low-grade dysplasia and the diagnostic distinction between these entities is challenging. Macroregenerative nodules were originally described as cirrhotic nodules distinctly larger than the surrounding nodules of cirrhosis [4]. Histologically these nodules contain portal tracts and the hepatocytes are identical to those of adjacent nodules [1]. Macroregenerative nodules are benign and not directly pre-neoplastic. Because of the difficulty in clearly defining a macroregenerative nodule versus a dysplastic nodule with low-grade dysplasia, some authors have recommended including macroregenerative nodules in the low-grade dysplastic nodule category [1]. However, the basic concept of a large, benign regenerative nodule without any atypia is clinically useful and still retains significant practical value. Dysplastic nodules are further divided into low- and high-grade dysplastic nodules based on histologic features.

6.2.2 Clinical Findings

As with large cell change and small cell change, macroregenerative and dysplastic nodules arise in the setting of chronic liver disease as a result of chronic inflammation, DNA damage and repair, and the subsequent accumulation of mutations. Dysplastic nodules are seen only in the setting of cirrhosis. Macroregenerative nodules are most commonly found in cirrhotic livers, but can rarely be seen following widespread parenchymal extinction from fulminant hepatitis.

Macroregenerative nodules and dysplastic nodules may be identified by imaging studies while screening for hepatocellular carcinoma. However, they typically do not show the high levels of hypervascular intensity on arterial phase of contrast imaging that is typical of hepatocellular carcinoma. Instead they are hypo- or isovascular in comparison to the background liver. In needle biopsy specimens, it can be helpful to know the imaging characteristics of the nodule. Subtle areas of hypervascularity on imaging may indicate progression of a dysplastic nodule to hepatocellular carcinoma.

Dysplastic nodules are associated with the development of hepatocellular carcinoma and their identification may increase screening frequency. One study carefully followed dysplastic nodules for a median of about two years and found that 46 % of dysplastic nodules disappeared, but 12 % progressed to hepatocellular carcinoma. Of note, another 24 % of individuals developed hepatocellular carcinoma, though not in the dysplastic nodule that was being followed [16].

Low-grade dysplastic nodules are typically followed clinically and radiographically and no immediate treatment is indicated. High-grade dysplastic nodules have a much greater risk for progression to hepatocellular carcinoma. The optimal treatment strategy for high-grade dysplastic nodules is the topic of active investigation, with the need to balance both the risk of overtreatment of lesions that will not progress and under treatment of lesions that are likely to progress. Some centers in the USA treat high-grade dysplastic nodules in the same way they treat well-differentiated HCC and may chemoembolize, radioablate, or transplant these lesions [17, 18].

6.2.3 Gross Findings

Macroregenerative nodules and dysplastic nodules are nodular lesions that differ in size, color, and consistency from the adjacent nodules of cirrhosis (Fig. 6.5). They may be single or multiple and typically measure greater than 1.5 cm. The lesions may bulge from the cut surface or may show a rust (iron), green (bile), or yellow (fat) cut surface. A “nodule within nodule” appearance may be seen grossly. In the setting of genetic hemochromatosis, the nodules can show less or absent iron, compared to the background liver, a finding called “iron free foci.”

Fig. 6.5 Dysplastic nodule. Grossly the lesion is distinctly different from the surrounding cirrhotic nodules, with respect to both size and color. This lesion was 1.1 cm in diameter

6.2.4 Microscopic Findings

Macroregenerative nodules have portal tracts and these portal tracts can show mild chronic inflammation and mild ductular reactions. The hepatocytes show no atypia and are identical cytologically to the hepatocytes in the rest of the liver. There may be fatty change when the rest of the liver also has steatosis.

Dysplastic nodules are identifiable at low power as nodules that are larger and distinctive compared to the other nodules of cirrhosis (Fig. 6.6). Low-grade dysplastic nodules typically show numerous portal tracts, some of which may be fibrotic and expanded. Unpaired arteries in the lobules are typically few or absent. The hepatic plate architecture is preserved at two cells thick. The cell density is mildly increased, but not to the same degree that is seen in hepatocellular carcinomas. Nuclei show mild atypia with nuclear enlargement and irregularities. Mitoses are infrequent. Pseudoglands are typically not seen, unless the liver as a whole is cholestatic. In addition, dysplastic nodules may have steatosis, but the fatty change is similar to that in the adjacent cirrhotic nodules. They do not have "nodule-in-nodule" morphology. Large cell change may be seen within or adjacent to low-grade dysplastic nodules.

High-grade dysplastic nodules show obvious cytological and architectural atypia but the changes fall short of malignancy (Fig. 6.7). In high-grade dysplastic nodules, the cell density is 1.5–2 times normal and there may be mild thickening of the hepatic plates, up to three cells thick. Portal tracks are commonly present within high-grade dysplastic nodules, but their numbers are typically fewer than in low-grade dysplastic nodules and macroregenerative nodules. Unpaired arteries can also be seen and the hepatocyte nuclei are atypical with hyperchromasia, and irregular contours. Small cell change may be present. A "nodule-in-nodule" appearance may be present, along with increased steatosis (when compared to adjacent cirrhotic nodules). Pseudoglands (Fig. 6.8) and an increased frequency of sinusoidal endothelization, demonstrated by diffuse CD34 staining, are often encountered. Reticulin stains often show subtle abnormalities, but do not show sufficient loss to diagnose hepatocellular carcinoma.

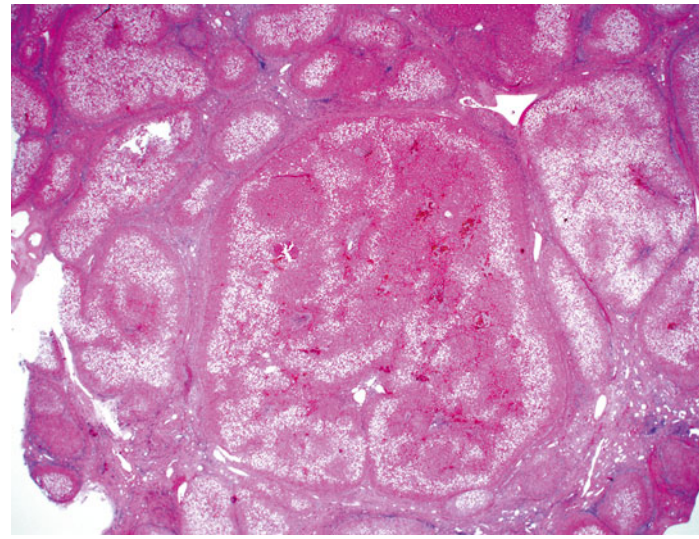

Fig. 6.6 Dysplastic nodule. The liver was cirrhotic due to nonalcoholic fatty liver disease and this lesion was distinct at the time of gross inspection. The presence of portal tracts with preserved hepatic plate architecture and only mild nuclear atypia are most consistent with a low-grade dysplastic nodule

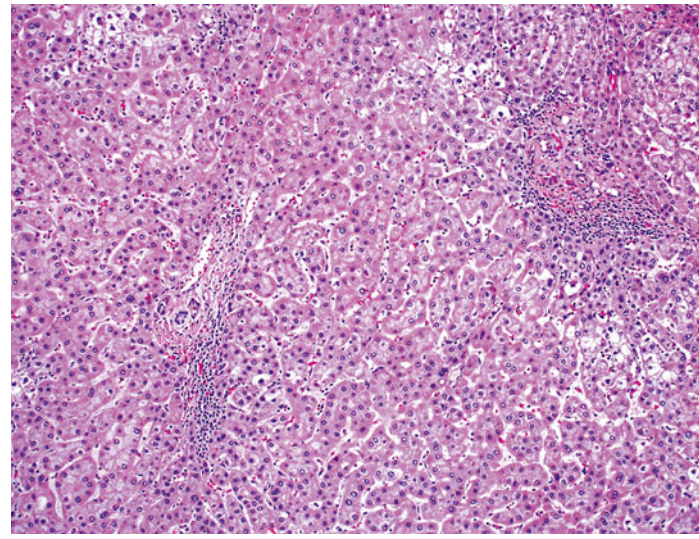

Fig. 6.7 High-grade dysplastic nodule. Note the increased nuclear-to-cytoplasmic ratio and the increased nuclear density. Two portal tracts are present and there are no unpaired arteries. Hepatic plates are slightly thickened but no more than three cells thick. No stromal invasion was identified at the septal interface zone. Despite some concerning features, there are insufficient findings for a definite diagnosis of hepatocellular carcinoma

6.2.5 Immunohistochemical Features

For difficult nodules that do not have sufficient atypia or reticulin loss to establish a diagnosis of hepatocellular carcinoma, immunohistochemical stains may be of help. The three most common immunohistochemical stains used to distinguish benign from malignant well-differentiated nodular lesions are glypican-3, glutamine synthetase, and heat shock protein-70 [19, 20]. Each of these markers is overexpressed in a percentage of hepatocellular carcinoma and the discriminating ability of any single marker is not optimal. However, when used as a panel, overexpression of two of the three markers in a given lesion is strongly

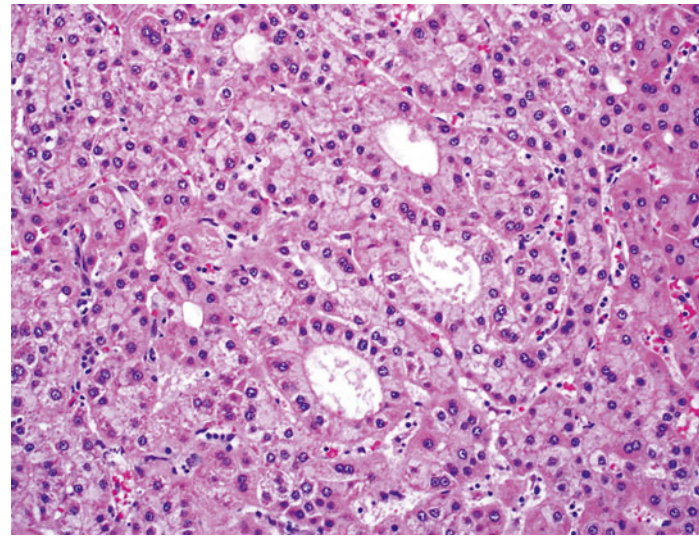

Fig. 6.8 Pseudoglands in a high-grade dysplastic nodule (same nodule as in Fig. 6.7)

associated with malignancy and may be used to support a diagnosis of hepatocellular carcinoma over high-grade dysplastic nodule. If only one marker is overexpressed, a diagnosis of high-grade dysplastic nodule can be favored.

Even if all of the stains are not available at your institution, using the ones available can still provide helpful supporting information, but the stains are best used in conjunction with the H&E findings and reticulin stains. Glutamine synthetase staining should be strong and diffuse to suggest hepatocellular carcinoma. Glypican-3 staining can be seen in some dysplastic nodules, so should not be used to diagnose hepatocellular carcinoma in isolation. Also of note, glypican-3 can also stain lipofuscin, so careful correlation with the H&E and immunostain result is needed to ensure glypican-3 staining is true positivity.

6.2.6 Differential Diagnosis

The key differential for a well-differentiated hepatic lesion in a cirrhotic liver includes a macroregenerative nodule, dysplastic nodule, and early hepatocellular carcinoma. The key distinguishing features are outlined in Table 6.1, but in essence a well-differentiate hepatocellular carcinoma is defined by the combination of architectural atypia, cytological atypia, and reticulin loss, while a macroregenerative nodule has hepatocytes that are cytologically identical to the rest of the liver, with no atypia and no reticulin loss. Dysplastic nodules fall in between, with varying degrees of cytological atypia.

Stromal invasion has been suggested as a histologic feature that improves the reproducibility of distinguishing dysplastic nodules from hepatocellular carcinoma. Stromal invasion is defined by cytologically atypical hepatocytes "invading" the stroma of portal tracts or fibrous bands. This finding is easiest to see in cases that are obviously hepatocellular carcinoma and more challenging to find on well-differentiated tumors. In addition, stromal invasion can be difficult to appreciate on H&E stains, even in obvious hepatocellular carcinomas. Immunostains for CK7 and CK19 can greatly aid in identifying stromal invasion, as a ductular reaction is absent at the edges of hepatocellular carcinoma nodules with

Table 6.1 Distinguishing features of hepatocellular nodular lesions in cirrhotic livers

Feature	Macro-regenerative nodule	Dysplastic nodule, low-grade	Dysplastic nodule, high-grade	Hepatocellular carcinoma
Imaging appearance	Hypo- or isovascular	Hypo- or isovascular	Hypo-, isovascular	Hypo-, iso-, or hypervascular
Gross appearance	Larger than adjacent nodules; often different than background liver in color	Distinctly different from adjacent cirrhotic nodules	Distinctly different from adjacent cirrhotic nodules	Distinctly different from adjacent cirrhotic nodules
Portal tracts	+	+	+	−
Thickened hepatic plates	−	−	± (up to 3 cells)	±
Nuclear hyperchromasia	−	−	±	±
Nuclear atypia	±	±	±	+
Cell density	Normal	Normal	1.5–2× normal	>2× normal
Nodule-in-nodule	Rare	Rare	±	±
Pseudoglands	Rare	Rare	±	±
Steatosis (strikingly more than background liver)	−	−	−	±
Unpaired arteries	±	±	±	+
Endothelization of sinusoids	−	−	±	+
Loss of reticulin	−	−	−	+
Stromal invasion	−	−	−	±
Two of three markers positive (glutamine synthetase, HSP-70, glypican-3)	−	−	−	±

stromal invasion, but present at the edges of high-grade dysplastic nodules (Fig. 6.9) [21].

A diagnosis of a high-grade dysplastic nodule should be made only when the histologic features are insufficient for an outright diagnosis of hepatocellular carcinoma. One study suggested a useful approach to adequacy and work-up criteria in the setting of hepatic nodules measuring between 1 and 2 cm, in the absence of clear radiologic evidence of malignancy [22]. To improve adequacy, they suggest needle biopsies be obtained from both the lesion and the adjacent non-lesional liver. In comparing the two biopsies, if they are the same and show only cirrhotic liver, this is not an adequate sampling. If the biopsy shows a nodule, the biopsy is adequate and an assessment of the degree of cytological changes (see Table 6.1) is performed. Some cases will be clearly macroregenerative nodules while others will be clearly hepatocellular carcinoma. For indeterminate lesions for which the differential is hepatocellular carcinoma or high-grade dysplastic nodule, immunostains for glypican-3, glutamine synthetase, and heat Shock Protein-70 can be helpful.

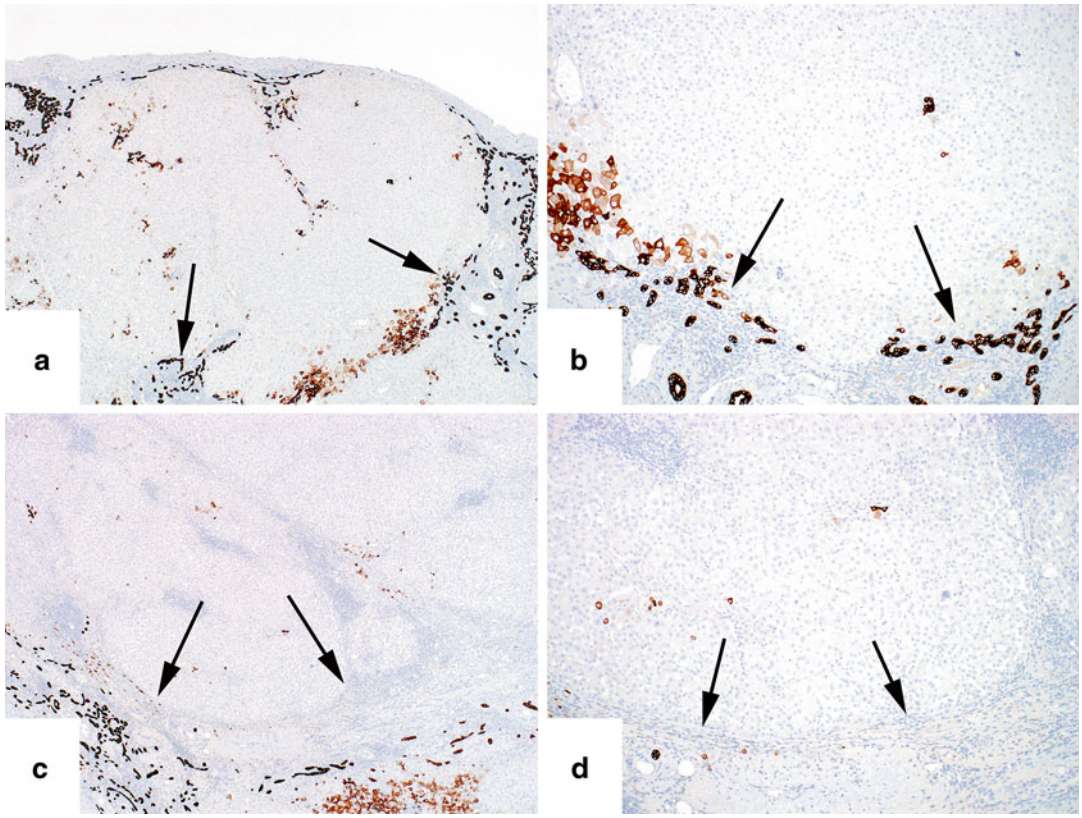

Fig. 6.9 Cytokeratin 7 immunostains for stromal invasion. Panels A and B are from a dysplastic nodule and the ductular reaction at the septal interface (*arrows*) is highlighted by cytokeratin 7 staining. In contrast, panels C and D are from an early hepatocellular carcinoma with stromal invasion, and demonstrate an absence of CK7 at the septal interface zone (*arrows*)

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