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Immunohistochemical pitfalls and the importance of glypican 3 and arginase in the diagnosis of scirrhous hepatocellular carcinoma

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Scirrhous hepatocellular carcinoma is a rare ill-defined morphological subtype of hepatocellular carcinoma characterized by marked stromal fibrosis. This variant can be difficult to distinguish from intrahepatic cholangiocarcinoma and metastatic adenocarcinoma, especially on needle biopsies. We performed immunohistochemistry for hepatocellular and adenocarcinoma-associated markers on 20 scirrhous hepatocellular carcinoma cases and compared the results with classical hepatocellular carcinoma and intrahepatic cholangiocarcinoma. Scirrhous hepatocellular carcinomas were significantly less likely to be HepPar-1 positive than classical hepatocellular carcinomas (26% and 74%, respectively; $P<0.001$) and were significantly more likely to express adenocarcinoma-associated markers such as epithelial cell adhesion molecule (63 *vs* 11%; $P<0.001$), cytokeratin 19 (26 *vs* 2%; $P<0.001$), and cytokeratin 7 (53 *vs* 2%; $P<0.001$). At least one of these adenocarcinoma-related markers was positive in 80% of scirrhous hepatocellular carcinoma cases. Glypican 3 and arginase were positive in 79% and 85% of cases of scirrhous hepatocellular carcinoma, respectively; the combined use of these two markers yielded 100% sensitivity for scirrhous hepatocellular carcinoma. In conclusion, the scirrhous morphology, absence of HepPar-1 staining, and frequent positivity with adenocarcinoma-related markers in scirrhous hepatocellular carcinoma can lead to an erroneous diagnosis of adenocarcinoma. Glypican 3 and arginase are the most reliable markers for identifying hepatocellular differentiation in this setting.
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Scirrhous hepatocellular carcinoma is a rare morphological subtype of hepatocellular carcinoma, comprising <5% of all hepatocellular carcinomas.[1–5] In addition to marked stromal fibrosis, scirrhous hepatocellular carcinomas are often characterized by subcapsular location, contiguous multinodularity, lack of encapsulation, lack of necrosis or hemorrhage, clear-cell change or hyaline bodies, and preserved intratumoral portal tracts.[2,3] Radiological studies in scirrhous hepatocellular carcinomas often show atypical findings such as peripheral enhancement in the arterial phase and persistent enhancement in the venous phase.[4,6]

Immunophenotypically, hepatocellular markers such as HepPar-1 are negative in the majority of scirrhous hepatocellular carcinomas, whereas markers such as cytokeratin 7 (CK7), which is typically associated with adenocarcinoma, are often positive,[2] thereby further confounding the diagnostic dilemma. The combination of atypical radiological features, abundant fibrous stroma, and aberrant immunohistochemical features can easily lead to a

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mistaken diagnosis of intrahepatic cholangiocarcinoma, metastatic adenocarcinoma, or combined hepatocellular-cholangiocarcinoma.

Distinction of scirrhous hepatocellular carcinoma from intrahepatic cholangiocarcinoma and metastatic adenocarcinoma has important therapeutic and prognostic implications. Long-term follow-up studies suggest that scirrhous hepatocellular carcinoma may be associated with similar or better prognosis than classical hepatocellular carcinoma.[2,4,5,7] In contrast, prognosis and survival of patients with intrahepatic cholangiocarcinoma is significantly worse than hepatocellular carcinoma.[5,8,9] Lymph node dissection is routinely performed during resection of intrahepatic cholangiocarcinoma but not hepatocellular carcinoma, as lymph node metastasis is relatively uncommon in the latter.[10,11] Treatment options for unresectable hepatocellular carcinoma include sorafenib and intra-arterial chemoembolization, whereas chemotherapeutic agents such as gemcitabine and fluoropyramidines are not beneficial.[12,13] In contrast, unresectable intrahepatic cholangiocarcinomas or resectable tumors with positive margins are treated with gemcitabine- or fluoropyramidine-based regimens.[14] Finally, orthotopic liver transplantation is established as a potentially curative option for selected cirrhotic patients with hepatocellular carcinoma who meet the Milan or extended UCSF criteria, whereas intrahepatic cholangiocarcinoma is considered a contraindication for orthotopic liver transplantation in most centers becasue of high recurrence rate and poor overall outcome.[15–18]

Despite the clinical relevance of accurate diagnosis, the utility of hepatocellular markers other than HepPar-1, such as polyclonal antibody against carcinoembryonic antigen (CEA) or expression of glypican 3 (GPC3) and arginase (ARG1), has not been studied in scirrhous hepatocellular carcinoma. Both HepPar-1 and polyclonal antibody against CEA show low sensitivity for poorly differentiated hepatocellular carcinoma.[19–22] In contrast, GPC3, an oncofetal antigen that is expressed in >80% of hepatocellular carcinomas, has high sensitivity for poorly differentiated hepatocellular carcinoma.[22–24] ARG1, a manganese metalloenzyme active in the urea cycle, has recently been identified as a sensitive and specific hepatocellular marker,[25] and also has not been evaluated in scirrhous hepatocellular carcinoma. Similarly, whereas CK7 positivity has been reported in scirrhous hepatocellular carcinoma,[2] the expression of other adenocarcinoma-associated markers, such as epithelial cell adhesion molecule (EPCAM) and cytokeratin 19 (CK19), has not been explored in scirrhous hepatocellular carcinoma. This study examines the immunophenotypic characteristics of scirrhous hepatocellular carcinoma using a variety of hepatocellular- and adenocarcinoma-associated markers, and compares the results with classical hepatocellular carcinoma and intrahepatic cholangiocarcinoma.

Materials and methods

The study population comprised 20 cases of scirrhous hepatocellular carcinoma and 16 cases of intrahepatic cholangiocarcinoma from the authors' institutions. Scirrhous hepatocellular carcinoma was defined as hepatocellular carcinoma with fibrous stroma comprising at least 50% of one low-power field. Scirrhous hepatocellular carcinoma cases included 11 resection specimens, 1 explant, and 8 core needle biopsies. In all biopsies, seven resection specimens and one explant specimen, the tumor showed abundant fibrous stroma that comprised >50% of the tumor. In four resection specimens, the scirrhous component was admixed with classical hepatocellular carcinoma. None of the cases had received preoperative chemotherapy or radiation. Cases of fibrolamellar carcinoma were excluded.

All specimens were fixed in 10% buffered formalin and embedded in paraffin. For immunohistochemistry, the following antibodies were used: hepatocyte-specific antigen (HepPar-1; dilution 1:200, clone OCH1E5; DAKO, Glostrup, Denmark), GPC3 (undiluted, clone 1G12; Biomosaics, Burlington, VT, USA), ARG1 (1:400 dilution, polyclonal; Sigma-Aldrich, St Louis, MO, USA), polyclonal antibody against CEA (1:20 dilution, polyclonal; DAKO, Glostrup, Denmark), CD117/c-kit (1:200 dilution; DAKO), CK7 (1:500 dilution, clone OV-TL 12/30; DAKO), CK19 (1:60 dilution, clone RCK108; DAKO), EPCAM (1:160 dilution, clone MOC-31; DAKO), and CD56 (1:100 dilution, clone 123C3; Zymed, San Francisco, CA, USA). DAKO 6.0 automated antigen retrieval system was used for all immunohistochemistry except ARG1 (ER1 antigen retrieval), CD56 (TRS 9.0 antigen retrieval), and CK19 (no antigen retrieval).

The intensity of immunohistochemical staining was scored as 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong). The number of positive cells was recorded as focal (<10%), patchy (10–50%), or diffuse (>50%). A score of at least 2+ staining in ≥10% of cells was regarded as positive. The results were compared with 169 cases of classical hepatocellular carcinoma (64 well differentiated, 91 moderately differentiated, and 14 poorly differentiated).[26] In some classical hepatocellular carcinoma cases, the available tissue was insufficient to perform all of the stains, and this is noted where applicable. The statistical analysis of differences in immunohistochemical staining patterns between groups was performed using Fisher's exact test and χ^2 test.

Results

Study Population

The age at diagnosis of scirrhous hepatocellular carcinoma patients ranged from 26 to 70 years (mean 56.5 years). There were 12 men and 8 women.

Cirrhosis was present in nine (45%) cases. Two patients each had chronic hepatitis B or C, and steatosis or steatohepatitis was seen in seven cases. Serum α-fetoprotein level was available in 12 cases at diagnosis and was elevated in six patients. Scirrhous hepatocellular carcinoma cases were enriched for background fatty liver disease compared with classical hepatocellular carcinoma (Table 1).

Hepatocellular Markers in Scirrhous and Classical HCC

In addition to fibrous stroma, all scirrhous hepatocellular carcinoma cases displayed at least focal 'hepatoid' morphology (polygonal tumor cells with moderate amounts of cytoplasm and lack of gland formation or mucin production; Figure 1). HepPar-1 was positive in 26% of scirrhous hepatocellular carcinoma compared with 74% of classical hepatocellular carcinoma ($P<0.001$; Table 2 and Figure 2). In the four cases of mixed scirrhous and classical hepatocellular carcinoma, both components were positive for HepPar-1 in one case, and both areas were negative for HepPar-1 in one case. In the remaining two cases with admixed features, HepPar-1 staining was seen in classical areas only (Figure 3). There was no significant difference in canalicular staining of polyclonal antibody against CEA in scirrhous and classical hepatocellular carcinoma (37% *vs* 54%, respectively; $P=0.223$).

GPC3 staining results were available in 19 scirrhous hepatocellular carcinoma cases. Of these, 15 (79%) were positive for GPC3, with strong diffuse staining in 9 (47%) tumors (Table 2 and Figure 2). The results were similar to classical hepatocellular carcinoma, of which 69% were positive for GPC3 ($P=0.440$). In the four cases of admixed scirrhous and classical hepatocellular carcinoma, both components were positive for GPC3 in two cases. In the other two cases, GPC3 was positive only in the scirrhous component (Figure 3).

ARG1 staining results were available in 13 scirrhous hepatocellular carcinoma cases. Of these, ARG1 was expressed in 11 (85%) cases, with strong diffuse staining in 9 (69%) cases (Table 2 and Figure 2). The results were comparable to classical hepatocellular carcinoma, which showed ARG1 expression in 95% of cases ($P=0.189$). In all four tumors with admixed scirrhous and classical hepatocellular carcinoma, ARG1 staining was positive in both components (Figure 3). All scirrhous hepatocellular carcinoma cases showed positive staining for either GPC3 and/or ARG1.

CK7, CK19, and EPCAM in Scirrhous and Classical HCC

CK7 and CK19 were positive in 53% and 26% of scirrhous hepatocellular carcinoma cases compared with 2% and 2% of classical hepatocellular carcinoma, respectively ($P<0.001$ for both; Table 2 and Figure 2). Staining for EPCAM yielded similar results (63% scirrhous hepatocellular carcinoma *vs* 11% classical hepatocellular carcinoma; $P<0.001$). Positive staining for at least one of these markers was seen in 80% of scirrhous hepatocellular carcinoma, whereas two or more markers were positive in 45% of cases. Among the 14 scirrhous hepatocellular carcinoma cases that were HepPar-1 negative, 64% expressed at least two adenocarcinoma-associated markers, and similar results were seen in 60% of CEA-negative scirrhous hepatocellular carcinoma.

Of the four cases showing admixed scirrhous and classical hepatocellular carcinoma, one case showed expression of CK7 only and another showed expression of both CK7 and CK19 in the scirrhous but not the classical component, whereas the other two cases expressed at least one of the adenocarcinoma-associated markers in both regions (Figure 3).

Immunophenotypic Profile of Intrahepatic Cholangiocarcinoma

All 16 (100%) cases of intrahepatic cholangiocarcinoma expressed CK7 and EPCAM, and 15 (94%) also expressed CK19 (Table 2). The results were significantly different from classical hepatocellular carcinoma (2%, 11%, and 2%, respectively; $P<0.001$ for each) as well as scirrhous hepatocellular carcinoma (53%, 63%, and 26%, respectively; Table 2). The numbers for scirrhous hepatocellular carcinoma are intermediate between intrahepatic cholangiocarcinoma and classical hepatocellular carcinoma. HepPar-1 and GPC3 positivity was seen

Table 1 Clinical features of scirrhous and classical hepatocellular carcinoma

	Gender (M:F)	*Mean age in years (range)*	*Hepatitis B (%)*	*Hepatitis C (%)*	*Steatosis or steatohepatitis (%)*	*Cirrhosis (%)*
Scirrhous hepatocellular carcinoma[a] ($n=20$)	1.5:1	56.5 (26–70)	12	12	41	45
Classical hepatocellular carcinoma[b] ($n=169$)	2.5:1	59.2 (11–85)	24	59	7	74[c]
P-value	0.306	0.400	0.362	<0.001	<0.001	0.016

[a]Information for cirrhosis risk factors available in 17 cases.
[b]Information for cirrhosis risk factors available in 129 cases.
[c]Information available in 163 cases.

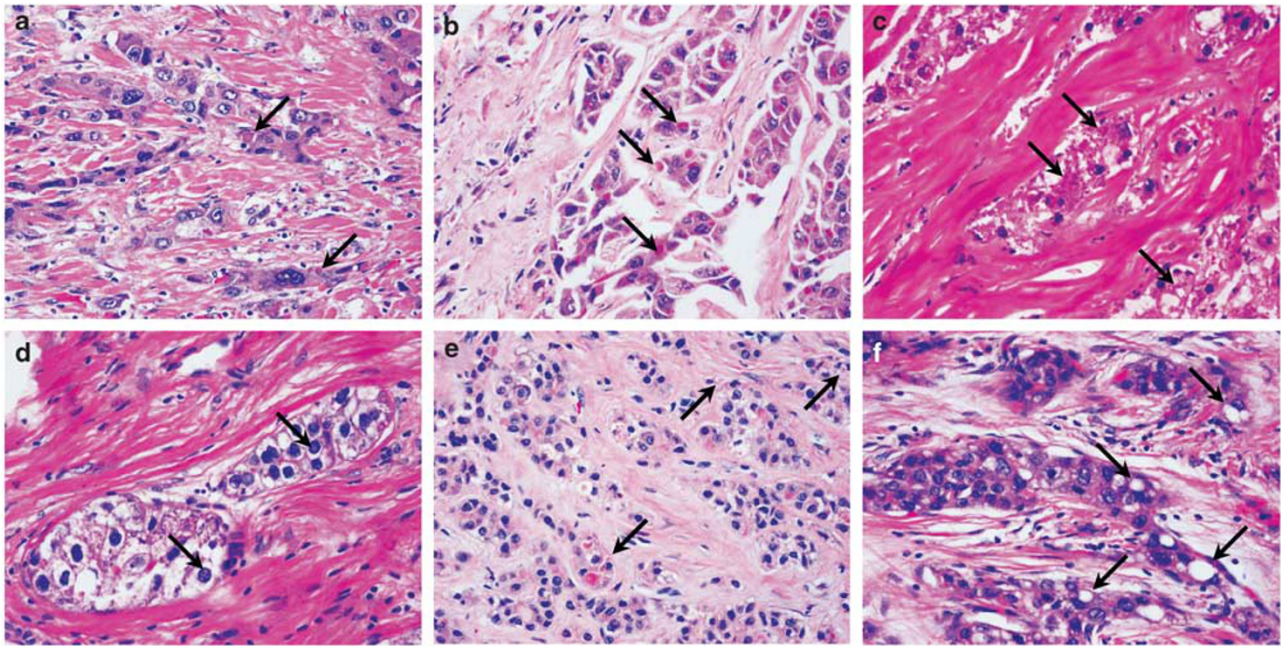

Figure 1 Morphological features of scirrhous hepatocellular carcinoma. Representative examples of scirrhous hepatocellular carcinoma illustrating the 'hepatoid' morphology (polygonal tumor cells with moderate cytoplasm), abundant fibrous stroma, and lack of glandular differentiation or mucin production (hematoxylin–eosin stain, ×400). Additional findings (eg, arrows) include intracytoplasmic eosinophilic globules (**a**, **b**), Mallory hyaline (**c**), intranuclear pseudoinclusions (**d**), bile (**e**), and cytoplasmic fat (**f**).

Table 2 Immunohistochemical staining of scirrhous hepatocellular carcinoma, classical hepatocellular carcinoma, and intrahepatic cholangiocarcinoma

	CEA[a]	*HepPar-1*	*GPC3*	*ARG1*	*CK7*	*CK19*	*EPCAM*
Scirrhous hepatocellular carcinoma ($n=20$)[b]	37	26	79	85	53	26	63
Classical hepatocellular carcinoma ($n=169$)[b]	54	74	69	95	2	2	11
Cholangiocarcinoma ($n=16$)[b]	0	7	6	0	100	94	100
P-value (scirrhous *vs* classical hepatocellular carcinoma)	0.223	<0.001	0.440	0.189	<0.001	<0.001	<0.001
P-value (scirrhous hepatocellular carcinoma *vs* cholangiocarcinoma)	0.026	0.209	<0.001	<0.001	0.001	0.001	0.011

Abbreviations: ARG1, arginase; CEA, polyclonal antibody to carcinoembryonic antigen; CK7, cytokeratin 7; CK19, cytokeratin 19; EPCAM, epithelial cell adhesion molecule; GPC3, glypican 3.
Numbers reflect percentages.
[a]Reflects canalicular staining with polyclonal antibody to CEA.
[b]In some cases, available tissue was insufficient to perform all stains. For classical hepatocellular carcinoma: CEA, $n=150$; GPC3, $n=153$; HepPar-1 and ARG1, $n=147$; CK7, $n=158$; CK19, $n=152$; EPCAM, $n=149$. For scirrhous hepatocellular carcinoma: CEA, HepPar-1, GPC3, CK7, CK19, and EPCAM, $n=19$; ARG1, $n=13$. For cholangiocarcinoma: CEA, $n=12$, HepPar-1, $n=14$.

in one case of intrahepatic cholangiocarcinoma each, whereas canalicular CEA and ARG1 were negative in all cases.

Progenitor Cell-Associated Markers in Scirrhous HCC

Immunohistochemistry for CD117/c-kit and CD56 was performed on a limited number of scirrhous hepatocellular carcinoma cases ($n=5$ and 4, respectively). One case showed focal weak CD117 staining, wheres CK7, CK19, EPCAM, and CD56 were negative in these areas. Patchy CD56 staining was seen in one other case, whereas CK7, CK19, EPCAM, and CD117 were negative in this case. Neither of these two cases showed small tumor cells at the periphery of the cell nests, and the CD117 and CD56 staining was observed in randomly distributed tumor cells (Figure 4a–c).

Discussion

The distinction of scirrhous hepatocellular carcinoma from intrahepatic cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma carries important prognostic implications and can influence the surgical approach, choice of chemotherapy, and decision to perform liver transplantation.[10–18] The World Health Organization (WHO) defines scirrhous hepatocellular carcinoma as a variant of hepatocellular carcinoma with marked sinusoidal fibrosis and atrophy of tumor trabeculae.[5] This description does not provide a precise definition of

scirrhous hepatocellular carcinoma. Most studies have used a fibrous stromal component of >50% to define scirrhous hepatocellular carcinoma, but other definitions, such as >25% fibrous stroma and/or a combination of gross and microscopic features, have been used.[1–4] Owing to the clinical implications involved, the correct categorization of this tumor as a hepatocellular carcinoma variant is more important than determining an exact cutoff for the amount of fibrous stroma requisite for the diagnosis.

Our study shows that the commonly utilized hepatocellular marker HepPar-1 has a significantly lower rate of positivity in scirrhous hepatocellular carcinoma compared with classical hepatocellular carcinoma. The low sensitivity of HepPar-1 in scirrhous hepatocellular carcinoma has been previously described in isolated reports,[27,28] as well as in two case series, in which sensitivities of 43 and 80% were reported, and were lower than classical hepatocellular carcinoma.[2,7] A canalicular pattern of staining with polyclonal antibody against CEA is considered a specific marker of hepatocellular differentiation but has low sensitivity.[19–21] Isolated case reports show variable expression in a limited number of scirrhous hepatocellular carcinoma cases.[27] In our study, canalicular CEA expression was seen in 37% and 54% of scirrhous and classical hepatocellular carcinomas, respectively. Its utility as a diagnostic marker in this context is therefore limited by the low sensitivity. In this respect, scirrhous hepatocellular carcinoma is similar to poorly differentiated hepatocellular carcinoma, where both HepPar-1 and CEA have relatively lower sensitivity. Our study also shows that the expression of adenocarcinoma-associated markers, such as CK7, CK19, and EPCAM, is significantly higher in scirrhous hepatocellular carcinoma compared with classical hepatocellular carcinoma. Matsuura *et al*[2] also reported positive CK7 staining in 71% of scirrhous hepatocellular carcinoma compared with 16% of classical hepatocellular carcinoma, although CK19 was negative in all

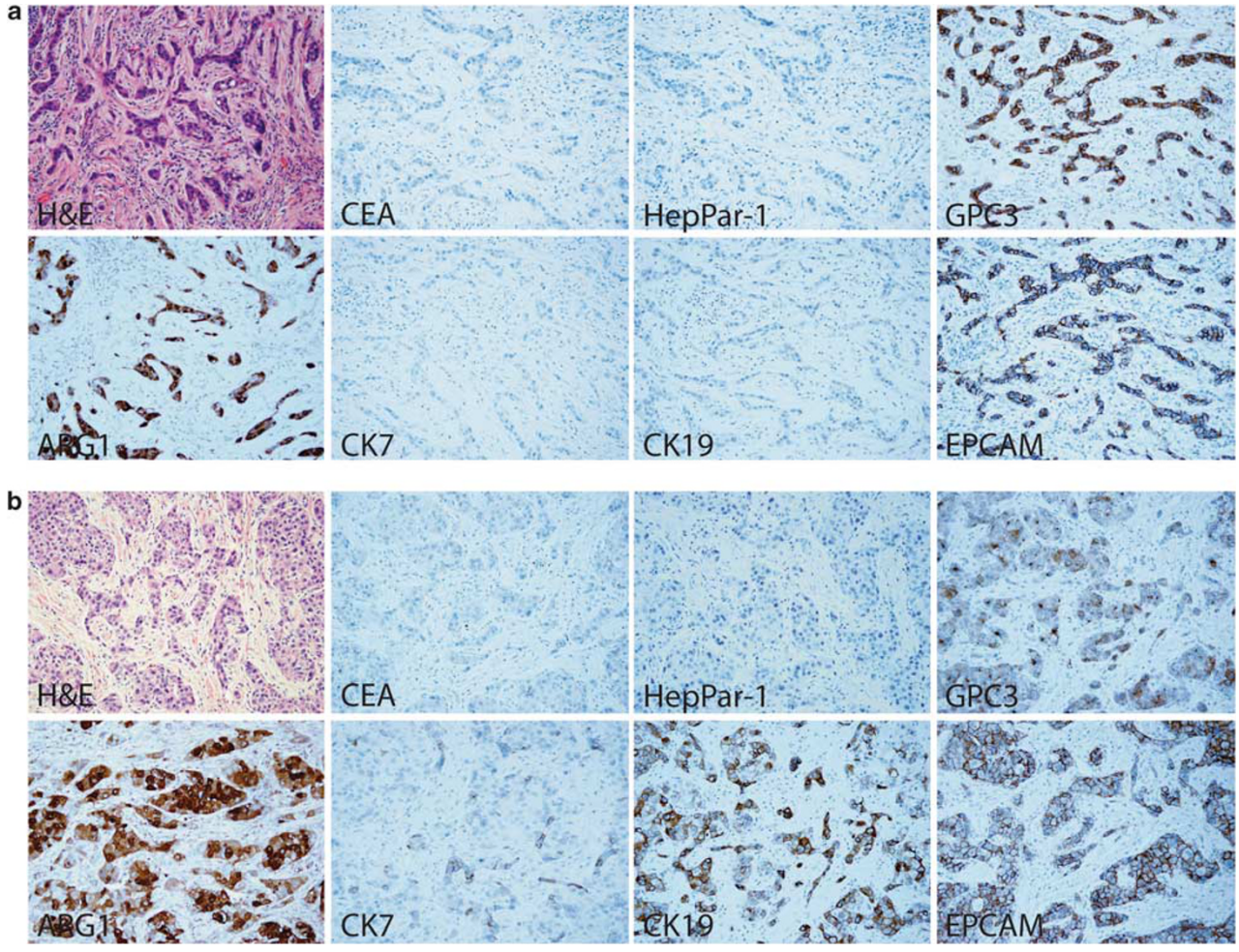

Figure 2 Immunohistochemical staining patterns of scirrhous hepatocellular carcinoma. (**a**) Positive staining for glypican 3 (GPC3), arginase (ARG1), and epithelial cell adhesion molecule (EPCAM); HepPar-1, carcinoembryonic antigen (CEA), cytokeratin 7 (CK7) and CK19 are negative (×200). (**b**) Positive staining for glypican 3, arginase, EPCAM, CK19, and CK7 (patchy); HepPar-1 and CEA are negative (×200). (**c**) Positive staining for arginase, CK7, glypican 3 (patchy), and EPCAM (patchy); HepPar-1, CEA, and CK19 are negative (×200). (**d**) Positive staining for glypican 3, arginase, and EPCAM; HepPar-1, CEA, CK7, and CK19 are negative (×200).

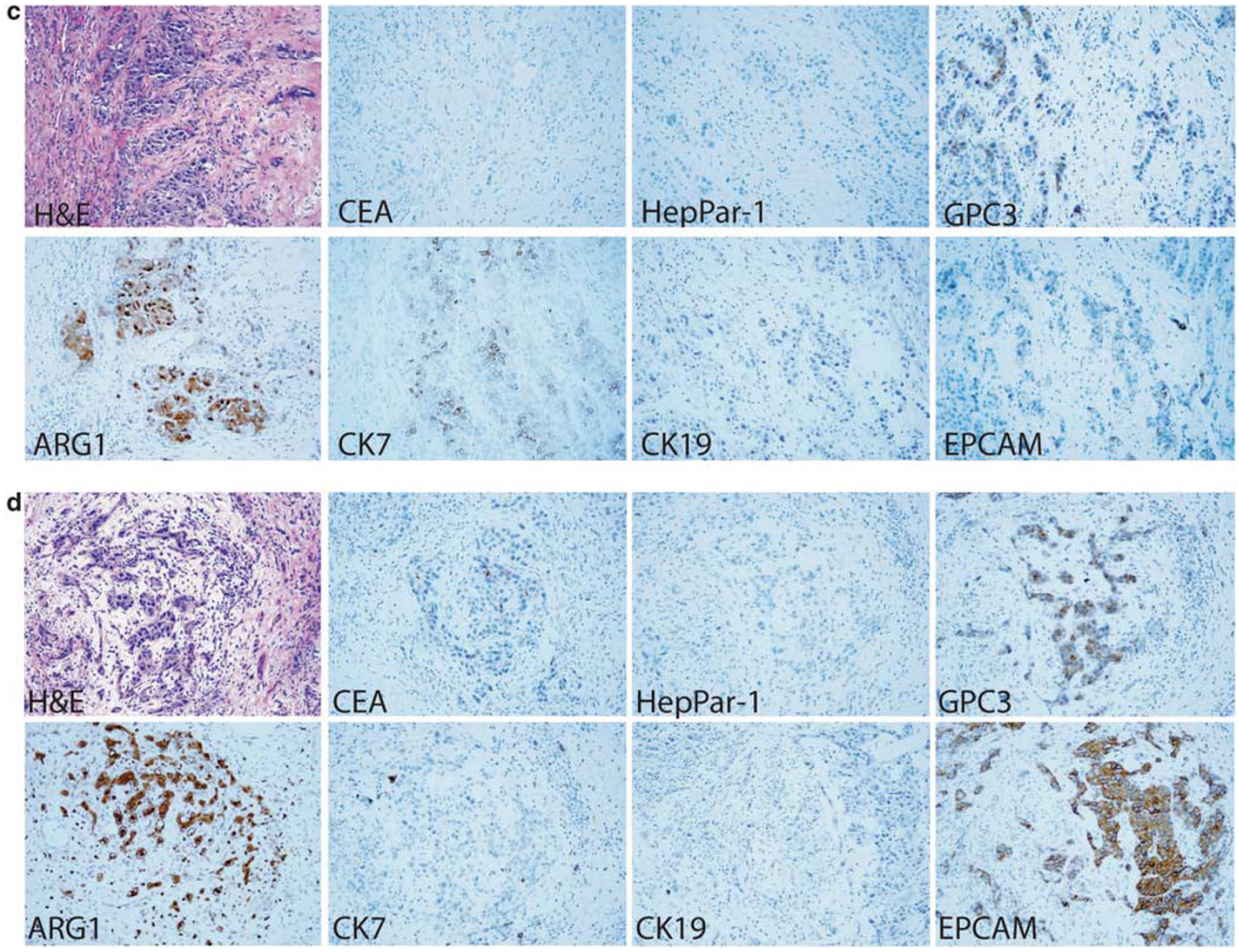

Figure 2 Continued.

scirrhous hepatocellular carcinoma cases in that study. Kurogi *et al*[3] also reported negative CK19 (but positive HepPar-1) staining in all scirrhous hepatocellular carcinomas in their study population, whereas Ariizumi *et al*[27] reported CK19 positivity in two scirrhous hepatocellular carcinoma cases. These differences may reflect differences in study populations and/or biological heterogeneity. CK7 and CK19 expression is less common in classical hepatocellular carcinoma and was expressed in 12% and 6% of cases, respectively, in a large study.[29]

While HepPar-1 and polyclonal antibody against CEA offer limited utility for diagnosis of scirrhous hepatocellular carcinoma, the role of more recently described hepatocellular markers, such as GPC3 and ARG1, has not been systematically examined. GPC3 is an oncofetal protein, which is expressed in >80% of hepatocellular carcinomas and has higher sensitivity than HepPar-1 and CEA for poorly differentiated hepatocellular carcinoma.[22–24,30–38] GPC3 is not a specific marker for hepatocellular differentiation and is expressed in a variety of other tumors, most of which do not share clinical or morphological resemblance to hepatocellular carcinoma.[39] Expression in cholangiocarcinoma has been described but is rare (<5% based on combined results of five studies including the present).[39–42] GPC3 expression was seen in 79% of scirrhous hepatocellular carcinoma and 6% (1 case) of intrahepatic cholangiocarcinoma in our study. Hence, GPC3 expression favors hepatocellular carcinoma over intrahepatic cholangiocarcinoma, even though it is not entirely specific in this context. ARG1 expression has higher sensitivity than HepPar-1 across the differentiation spectrum of hepatocellular carcinoma.[25] Arginase staining may rarely be seen in other tumors but has not been described in cholangiocarcinoma.[25,43] Positive arginase staining was observed in 85% of scirrhous hepatocellular carcinomas and none of the cholangiocarcinomas in our study. Notably, the combined use of GPC3 and arginase identified all cases of scirrhous hepatocellular carcinoma.

The gene expression pattern in scirrhous hepatocellular carcinoma has similarities to intrahepatic cholangiocarcinoma.[44] Along with the expression of adenocarcinoma-associated markers and concomitant absence of hepatocellular markers such as HepPar-1 and CEA, this may raise the argument that

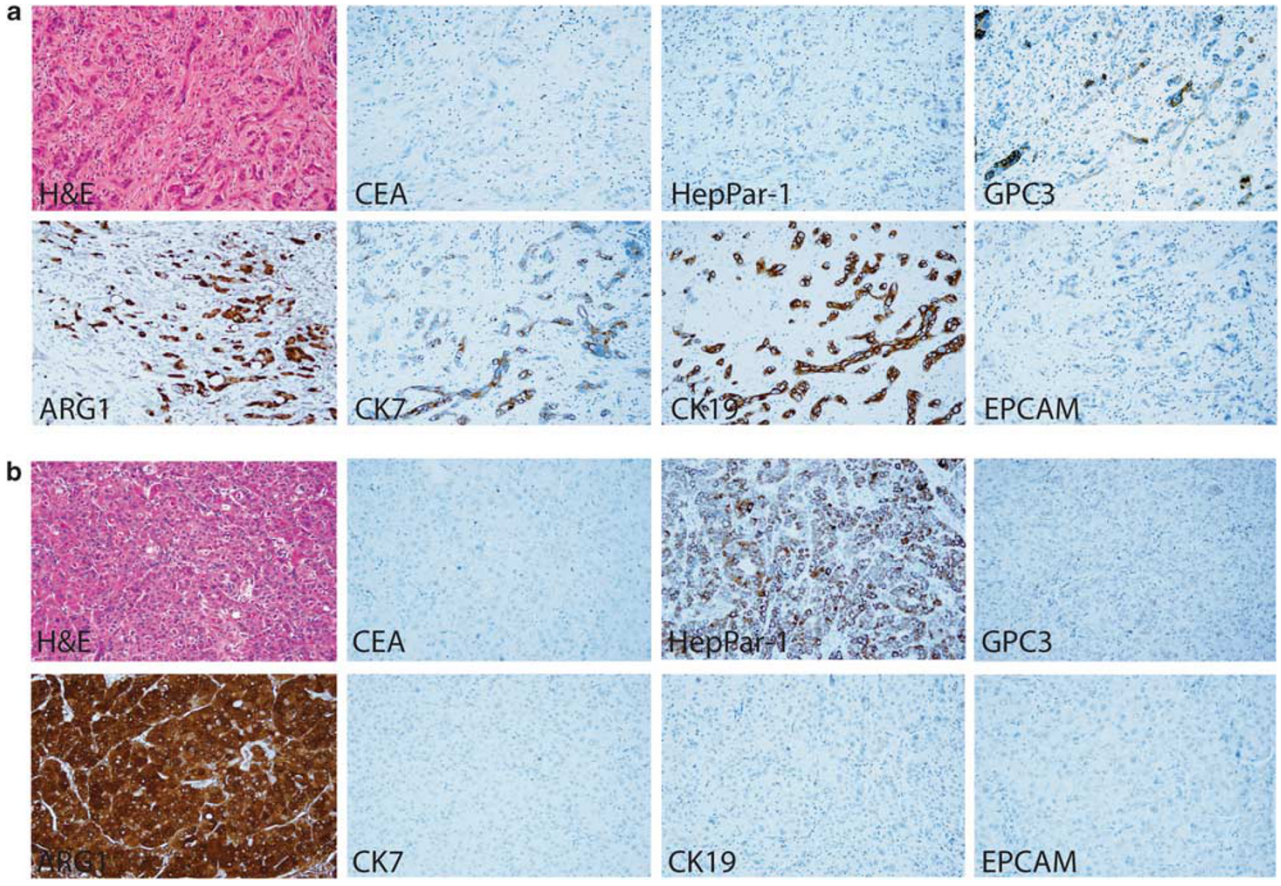

Figure 3 Immunohistochemical staining patterns of admixed scirrhous—classical hepatocellular carcinoma. The scirrhous hepatocellular carcinoma portion (**a**) is negative for HepPar-1 and positive for glypican 3 (GPC3), cytokeratin 7 (CK7), and CK19, whereas classical hepatocellular carcinoma (**b**) is positive for HepPar-1 and negative for glypican 3, CK7, and CK19. Arginase (ARG1) is expressed in both areas, while carcinoembryonic antigen (CEA) and epithelial cell adhesion molecule (EPCAM) are negative in both components.

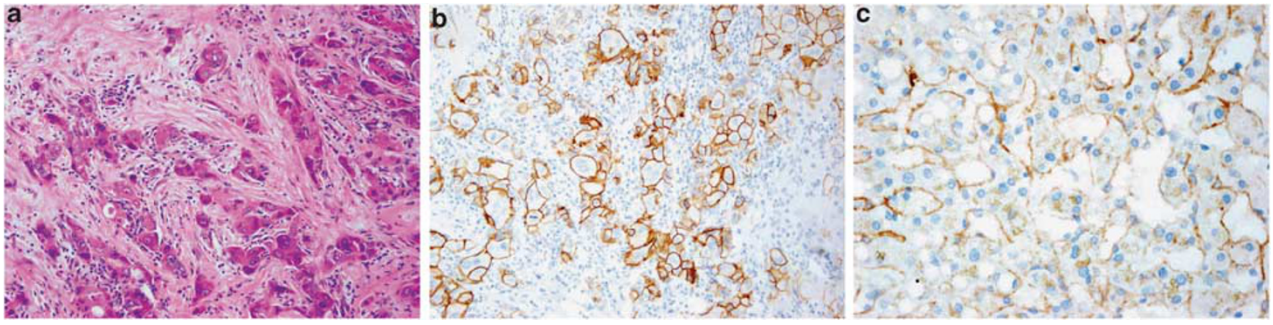

Figure 4 Lack of stem or progenitor cell features in scirrhous hepatocellular carcinoma. (**a**) Scirrhous hepatocellular carcinoma (hematoxylin–eosin stain, ×400). (**b**) Immunohistochemical stain for CD56 shows focal membranous staining of large neoplastic cells without evidence of small, peripheral progenitor, or stem cell-like cells. (**c**) Immunohistochemical stain for CD117, showing focal weak membranous staining of large neoplastic cells. Both stains were positive in a small minority of tumor cells.

the cases in our series represent intrahepatic cholangiocarcinoma. However, this premise is not supported by morphological and overall immunohistochemical results. All scirrhous hepatocellular carcinoma cases in our study showed distinct 'hepatoid' morphology and lacked discrete gland formation or mucin production. The expression of GPC3 and/or ARG1 in all the cases also supports the diagnosis of hepatocellular carcinoma.

In some reports, a subpopulation of small tumor cells has been observed at the periphery of the cell nests in scirrhous hepatocellular carcinoma, and it has been postulated that these are likely to represent hepatic stem cells or progenitor cells. In the study by Fujii *et al*,[45] the expression of CK7, CK19, and EPCAM was predominantly observed in these small, peripheral tumor cells, whereas the majority of tumor cells were negative for these markers.

These cases would correspond to the classical subtype of combined hepatocellular-cholangiocarcinoma with stem cell features in the new WHO classification.[5,46] In our study, none of the cases showed small tumor cells at the periphery. The staining for CK7, CK19, and EPCAM in our cases was distributed in a patchy or diffuse manner and not restricted to the periphery of tumor cell nests. According to the new WHO classification, the intermediate subtype of combined hepatocellular-cholangiocarcinoma with stem cell features also shows abundant fibrous stroma and may closely resemble scirrhous hepatocellular carcinoma.[5,47] In our cases, the tumor cells did not show morphological features typically associated with progenitor cells, such as small size, oval shape, and scanty cytoplasm. The progenitor-cell-related markers performed on a limited number of cases were also largely negative, further arguing that our cases do not fulfill the criteria for combined hepatocellular-cholangiocarcinoma with stem cell features.[5,45] CK19 and EPCAM have been considered 'stemness-related' markers in hepatocellular carcinoma.[5,44,46–48] Although we found high expression of these markers in scirrhous hepatocellular carcinoma, the absence of other 'stemness-related' morphological and immunohistochemical features does not support this interpretation in our series. The significance of this apparent heterogeneity of scirrhous hepatocellular carcinoma across various studies is unclear, but may represent different tumor subgroups and therefore reflect underlying differences in carcinogenetic processes in hepatocellular tumors with abundant fibrous stroma.

Hepatocellular carcinoma with expression of CK7, CK19, and EPCAM has been associated with a poor outcome.[48–50] As most scirrhous hepatocellular carcinomas express one or more of these markers, it would be expected that scirrhous hepatocellular carcinoma is more aggressive compared with classical hepatocellular carcinoma. However, the outcome of scirrhous hepatocellular carcinoma has been variably reported as better, similar, or worse than classical hepatocellular carcinoma in different studies.[2,4,5,7] Low numbers of cases and variability in the criteria of diagnosis of scirrhous hepatocellular carcinoma may be responsible for these differences. The low incidence of lymph node metastasis in scirrhous hepatocellular carcinoma compared with intrahepatic cholangiocarcinoma suggests that the biological behavior of scirrhous hepatocellular carcinoma may more closely resemble classical hepatocellular carcinoma.[44] In addition to these prognostic implications, the inclusion of a cholangiocarcinoma component in the diagnosis can lead to clinical consequences such as use of a gemcitabine-based regimen and denial of transplantation. Until the clinical outcome and therapeutic options in hepatocellular tumors with fibrous stroma are better established, it is prudent to separate scirrhous hepatocellular carcinoma from cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma (classical type as well as the type with stem cell features) and rely on the traditional criteria of well-formed glands and mucin production for the diagnosis of a cholangiocarcinoma component. As shown in this and other studies, expression of EPCAM, CK7, and CK19 does not constitute sufficient evidence of biliary differentiation.

In summary, the presence of abundant fibrous stroma, negative HepPar-1 staining, and frequent expression of CK7 and CK19 in scirrhous hepatocellular carcinoma can easily be mistaken for intrahepatic cholangiocarcinoma or metastatic adenocarcinoma. ARG1 and GPC3 are the preferred hepatocellular markers in this morphological setting, and their combined use yielded 100% sensitivity for scirrhous hepatocellular carcinoma in this series.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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