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Original contribution

Steatotic and nonsteatotic scirrhous hepatocellular carcinomas reveal distinct clinicopathological features☆

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Summary We investigated the clinicopathological and molecular characteristics of scirrhous hepatocellular carcinoma (HCC) to elucidate its uniqueness. Samples from 120 resected HCC cases underwent immunohistochemical analysis. Tumor area containing fibrous stroma and the percentage of steatotic cells within the tumor were evaluated. In our previous report, tumors were immunohistochemically subclassified as biliary/stem cell markers–positive (B/S) (cytokeratin 19 and/or sal-like protein 4 and/or epithelial cell adhesion molecule positive), Wnt/β-catenin signaling–related markers–positive (W/B) (β-catenin and/or glutamine synthetase positive), or all markers–negative (−/−) groups. Thirty-seven cases (31%) with fibrous stroma making up ≥50% of the largest tumor area were defined as scirrhous HCC (sHCC); the other 83 cases (69%) were categorized as common HCC (cHCC). Clinicopathologically, sHCC had fewer poorly differentiated tumors ($P = .037$) and a higher percentage of cases with steatosis ($P = .025$) than cHCC. sHCC cases were further divided into two subgroups: those with ≥5% steatotic cells (steatotic sHCC) and those with <5% steatotic cells (nonsteatotic sHCC). Hepatitis B virus infection was more frequent in nonsteatotic sHCC ($P = .029$), and non-B, non-C cases were more frequent in steatotic sHCC ($P = .006$). Steatotic sHCC tended to have a longer time to recurrence than nonsteatotic sHCC and cHCC. Most nonsteatotic sHCC cases belonged to B/S group, whereas most steatotic sHCC belonged to −/− group. The same tendency in sHCC was shown in another cohort. Distinct features were seen in steatotic and nonsteatotic sHCC, and both sHCC subgroups exhibited different clinicopathological and molecular features from cHCC. These findings support the hypothesis that sHCC is an independent entity.

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1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer worldwide [1]. The majority of HCC cases arise from chronic infection of hepatitis viruses, alcohol overconsumption, or nonalcoholic steatohepatitis. Most commonly, the cut surface of HCC reveals a round nodule with an expansive growth pattern and a circumscribing fibrous capsule. The histological pattern is trabecular growth of atypical liver cells without involvement of fibrous stroma. About 5% of HCCs are a rare variant named scirrhous HCC [1]. Scirrhous HCC is considered to be the same entity that was previously referred to as sclerosing hepatic carcinoma [2-4] or scalloped tumor [5]. Its distinct features include an irregular cut surface without bulging and with no fibrous capsule formation. Histologically, scirrhous HCC contains abundant fibrous stroma as thick bands that surround the tumor islands and/or fine fiber infiltrating the tumor trabeculae. Although the histological features of scirrhous HCC are apparently different from those of common HCC, it is not currently recognized as an independent entity.

Recently, investigators have used gene expression profiling to establish molecular classifications of HCC, for example, types S1–S3 [6], types G1–G6 [7], or proliferation/non-proliferation classes [8] in an attempt to classify and group its biological behavior. We previously reported new subclassifications of HCC using molecular markers related to HCC tumor aggressiveness and prognosis (ie, cytokeratin 19 [CK19], Sal-like protein 4 [SALL4], epithelial cell adhesion molecule [EpCAM], β-catenin, and glutamine synthetase [GS]). By applying immunohistochemical analysis of the above markers, we classified HCC into three groups: biliary/stem cell markers–positive (B/S) group, Wnt/β-catenin signaling–related markers–positive (W/B) group, and all negative (−/−) group [9].

To investigate the clinicopathological and molecular characteristics of scirrhous HCC, we assessed the area containing fibrous stroma within the entire section of tumor nodules. This allowed us to define scirrhous HCC and to carry out relevant clinicopathological investigations.

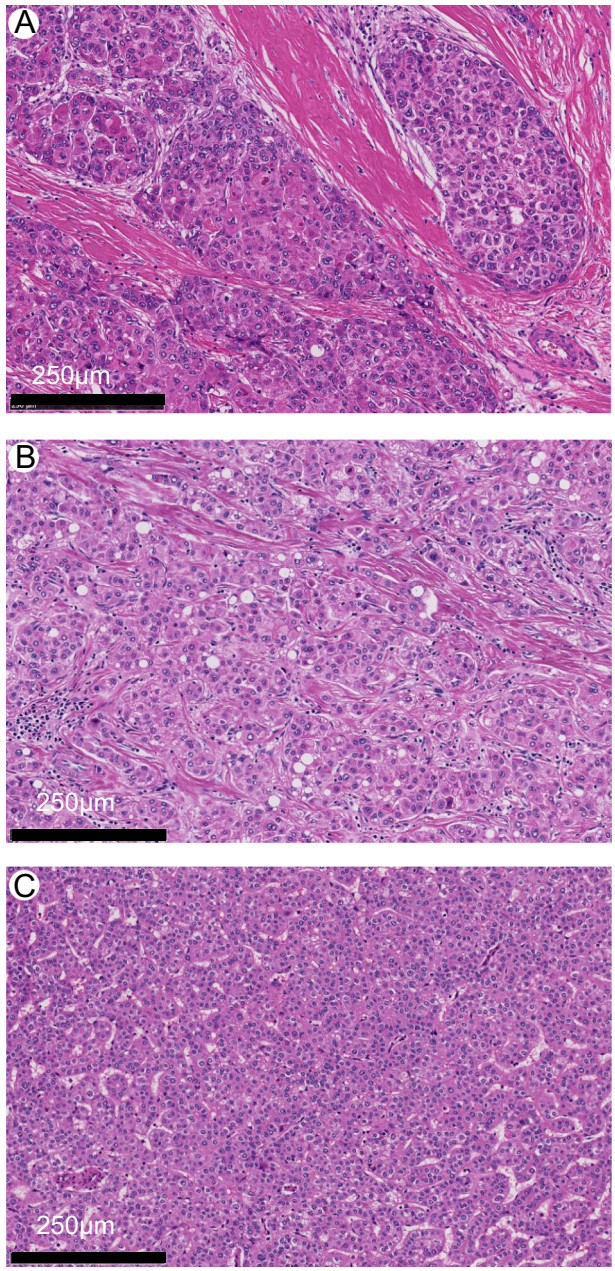
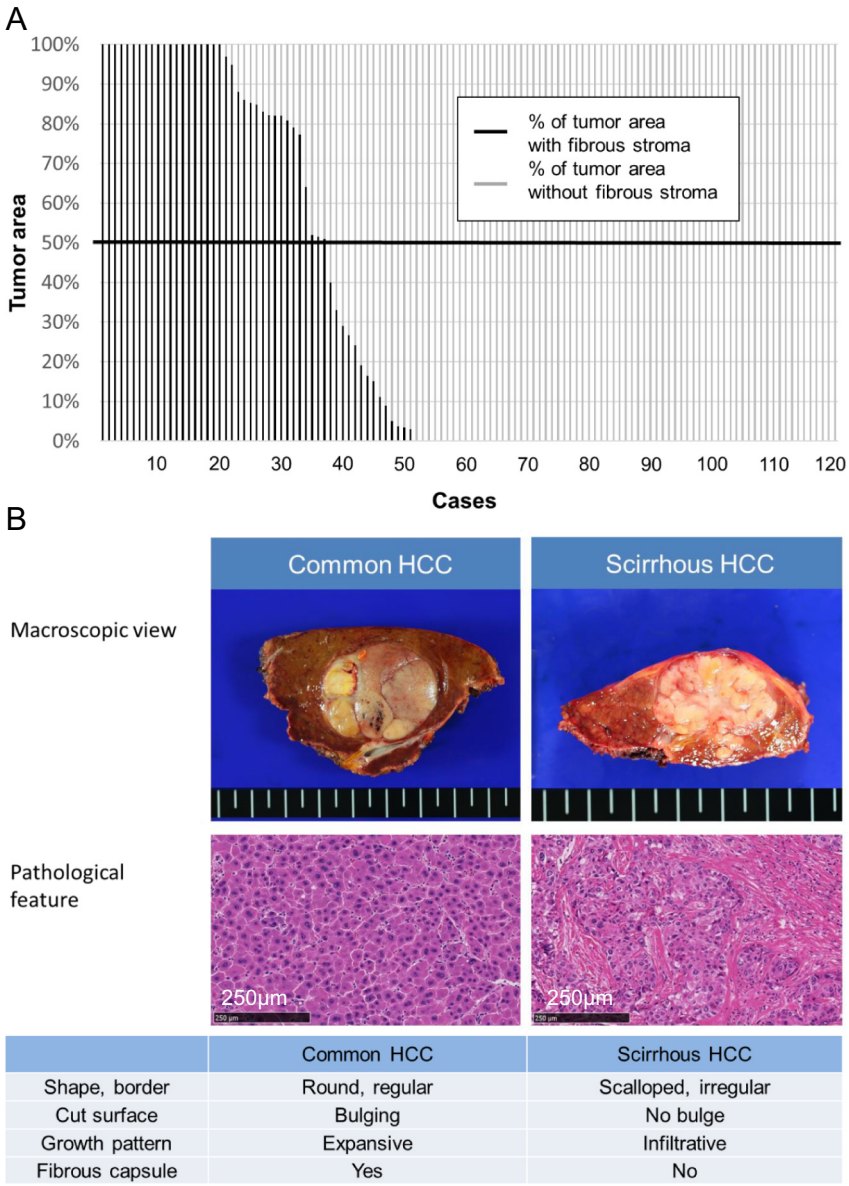


Fig. 1 Histological features of HCCs both with and without tumor areas containing fibrous stroma. A, Tumor area with thick fibrous bands surrounding tumor islands. B, Tumor area with fine fibers infiltrating the tumor trabeculae. A and B are classified as tumor areas with fibrous stroma. C, Tumor area without the characteristics explained in A and B were classified as tumor areas without fibrous stroma. (A-C, Hematoxylin and eosin stain.) Scale bars: 250 μm.

2. Materials and methods

2.1. Case selection and etiology

In total, 231 patients who underwent surgical resection of HCC at Keio University Hospital from 2003 to 2015 were reviewed. Patients who had undergone transplantation were included in this review. From these cases, further selection was made according to the following criteria: (1) primary and solitary tumor, (2) no preoperative anticancer therapy administered, and (3) full paraffin sections available for the largest tumor area and of a quality adequate for histological assessment. Finally, 120 cases were selected, and the patients' medical records were reviewed. Data relating to patients' etiology, age, sex, tumor size, viral infection status, alcohol intake, and diabetes mellitus status were obtained from clinical records. Hepatitis B virus (HBV) infection was determined using hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) infection was determined using anti-hepatitis C virus antibody (HCV-Ab). Sixty-nine of the patients included in the current study (who underwent surgical resection of HCC at Keio University Hospital



	Common HCC	Scirrhous HCC
Shape, border	Round, regular	Scalloped, irregular
Cut surface	Bulging	No bulge
Growth pattern	Expansive	Infiltrative
Fibrous capsule	Yes	No

Fig. 2 Evaluation of HCC containing tumor area with fibrous stroma and its characteristics. **A,** Bar graph showing the percentage of tumor area with and without fibrous stroma in the 120 HCC cases under consideration. The cases could be meaningfully categorized into two groups with a cut-off value of 50% tumor area with fibrous stroma. **B,** Macroscopic and histological features and characteristics of common HCC and scirrhous HCC.

from 2003 to 2010) were also included among the 142 patients investigated in our previous study [9].

Additionally, a validation analysis was carried out in which 374 resected HCC cases from the National Cancer Center, Tokyo, operated on between 2001 and 2006, were reviewed pathologically.

Informed consent was obtained from patients from both facilities.

2.2. Histological evaluation

Specimens from all 120 HCC cases were fixed in 10% formalin immediately after resection, embedded in paraffin, and cut into thin slices for staining. Hematoxylin and eosin staining was performed subsequently.

The area containing fibrous stroma within the tumor nodule was evaluated as follows. (1) The entire section containing the largest tumor area was histopathologically evaluated and classified into areas with or without fibrous stroma. Fibrous stroma was defined as thick fibrous bands surrounding the tumor islands and/or fine fibers infiltrating the tumor trabeculae (Fig. 1). Histological evaluations were performed independently by two pathologists (MH and HO). (2) The areas of the two components were plotted onto macroscopic images of the tumor. (3) The areas of the plotted regions were measured using Image J software 1.48v (National Institutes of. Health, Bethesda, MD, USA), and the

Table 1 Univariate analysis of clinicopathological parameters associated with sHCC and cHCC

		sHCC (n = 37)		cHCC (n = 83)		χ^2 Test *P*
Age	≥67 y [a]	24	64.9%	50	60.2%	.630
	<67 y	13	35.1%	33	39.8%	
Sex	Male	31	83.8%	69	83.1%	.930
	Female	6	16.2%	14	16.9%	
Tumor size	≥3.8 cm [b]	8	21.6%	38	45.8%	.012
	<3.8 cm	29	78.4%	45	54.2%	
Hepatitis B virus	Positive	7	18.9%	14	16.9%	.785
	Negative	30	81.1%	69	83.1%	
Hepatitis C virus	Positive	9	24.3%	32	38.6%	.129
	Negative	28	75.7%	51	61.4%	
Non-B, non-C	Non-B, non-C	21	56.8%	37	44.6%	.218
	Others	16	43.2%	46	55.4%	
Background liver inflammation	Normal liver, chronic hepatitis	26	70.3%	48	57.8%	.196
	Precirrhosis, liver cirrhosis	11	29.7%	35	42.2%	
Tumor grade	Well differentiated	4	10.8%	15	18.1%	.037
	Moderately differentiated	32	86.5%	54	65.1%	
	Poorly differentiated	1	2.7%	14	16.8%	
Portal vein invasion and/or intrahepatic metastasis	Positive	20	54.1%	37	44.6%	.337
	Negative	17	45.9%	46	55.4%	
Venous invasion	Positive	2	5.4%	6	7.2%	1.000 [c]
	Negative	35	94.6%	77	92.8%	
Bile duct invasion	Positive	1	2.7%	2	2.4%	1.000 [c]
	Negative	36	97.3%	81	97.6%	
Tumor staging	Stage I	19	51.4%	43	51.8%	.963
(TNM)	Stage II [d]	18	48.6%	40	48.2%	
Tumor cell steatosis	≥5%	21	56.8%	29	34.9%	.025
	<5%	16	43.2%	54	65.1%	
Mallory-Denk bodies	+	21	56.8%	23	27.7%	.002
	−	16	43.2%	60	72.3%	
Tumor cell ballooning	+	29	78.4%	41	49.4%	.003
	−	8	21.6%	42	50.6%	
Inflammatory cell infiltration	+	33	89.2%	45	54.2%	<.001
	−	4	10.8%	38	45.8%	
Steatohepatitic features [e]	+	27	73.0%	23	27.7%	<.001
	−	10	27.0%	60	72.3%	
Background liver steatosis	≥5%	29	78.4%	48	57.8%	.030
	<5%	8	21.6%	35	42.2%	
Alcohol intake [f]	+	3	8.1%	6	7.2%	1.000 [c]
	−	34	91.9%	77	92.8%	
NASH	NASH	5	13.5%	2	2.4%	.028 [c]
	others	32	86.5%	81	97.6%	
ASH	ASH	2	5.4%	6	7.2%	1.000 [c]
	others	35	94.6%	77	92.8%	
Diabetes mellitus	+	12	32.4%	23	27.7%	.599
	−	25	67.6%	60	72.3%	

Abbreviations: sHCC, scirrhous hepatocellular carcinoma; cHCC, common hepatocellular carcinoma; NASH, nonalcoholic steatohepatitis; ASH, alcoholic steatohepatitis.

[a] Average age of sHCC + cHCC patients was 67 years.

[b] Average tumor size of sHCC + cHCC cases was 3.8 cm. The range for sHCC was 0.7–7.0 cm, and the range for cHCC was 1.0–17.5 cm.

[c] Using Fisher's direct method.

[d] One stage IVa cHCC case was included.

[e] sHCC fulfilling three of the following four criteria: steatosis, Mallory-Denk bodies, tumor cell ballooning, and inflammatory cell infiltration.

[f] Intake of ≥60 g (male) or ≥ 40 g (female) alcohol per day.

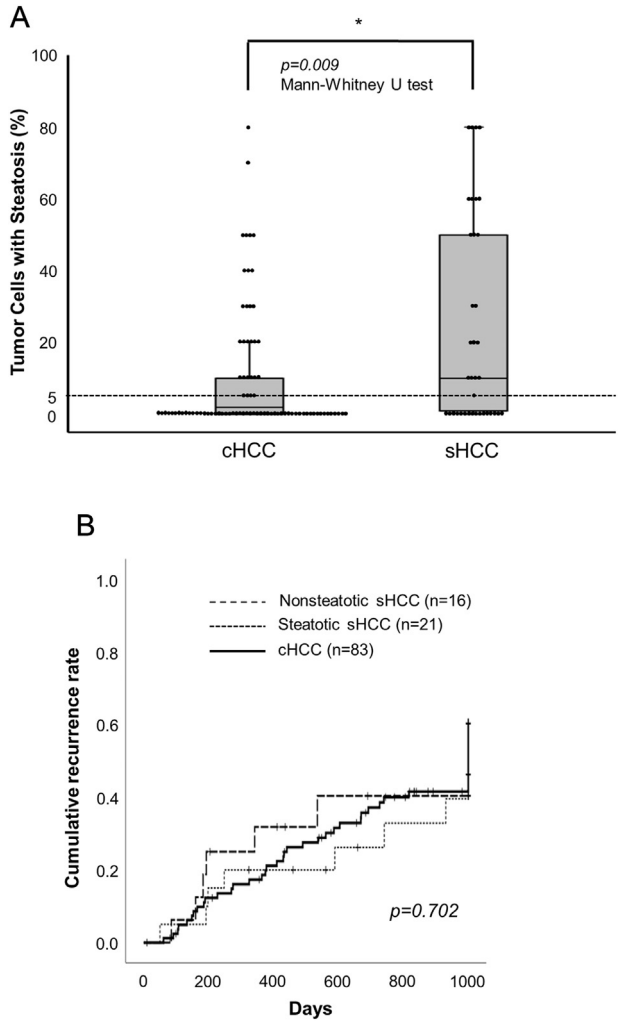


Fig. 3 Percentage of tumor cells with steatosis and cumulative recurrence rates of sHCC and cHCC. **A,** Beeswarm dot plot and box-and-whisker plot showing the distribution of the percentage of tumor cells with steatosis in cHCC and sHCC. The distribution of steatosis in sHCC was significantly wider than that in cHCC. **B,** Cumulative recurrence rates of nonsteatotic sHCC, steatotic sHCC and cHCC. The differences between the three groups were not significant. However, steatotic sHCC tended to have a better prognosis than the other two groups.

percentages of the areas with and without fibrous stroma were used for evaluation.

Factors that were analyzed for association with the tumor area containing fibrous stroma included background liver inflammation, tumor grade, portal vein invasion and/or intrahepatic metastasis (vp/im), venous invasion, bile duct invasion, tumor staging (TNM), tumor cell steatosis, Mallory-Denk bodies, tumor cell ballooning, inflammatory cell infiltration, background liver steatosis, nonalcoholic steatohepatitis (NASH), and alcoholic steatohepatitis (ASH). For each case, the percentage of tumor cells with steatosis and the level of background liver steatosis were also evaluated according to the nonalcoholic steatohepatitis/nonalcoholic fatty liver disease (NASH/NAFLD) criteria [10]. The percentage of normal hepatocytes with large or small lipid droplets in the cytoplasm was assessed by measurements in both low- (×4) and high- (×10–20) resolution images. The following definitions were used for NASH and ASH: NASH, ≥5% background liver steatosis + hepatocellular ballooning + lobular inflammation + non-B, non-C + <30 g/day (male) or <20 g/day (female) alcohol consumption [10,11]; ASH, ≥5% background liver steatosis + non-B, non-C + ≥60 g/day (male) or ≥40 g/day (female) alcohol consumption. The factor "Steatohepatitic features" within the tumor area was considered positive when three of the following four criteria were fulfilled: steatosis, Mallory-Denk bodies, tumor cell ballooning, and inflammatory cell infiltration [12].

2.3. Immunohistochemical evaluation

Immunohistochemical staining of specimens from all 120 HCC cases was performed using a Bond-Max automated immunohistochemical staining machine (Leica Microsystems, Milton Keynes, UK) according to the manufacturer's instructions. The antibodies used in this analysis are listed in Supplementary Table 1, and the evaluation was done according to the procedures described previously [9] (Supplementary Table 2).

2.4. Statistical analysis

The differences between two groups were statistically analyzed using the chi-square test, Fisher's direct method or the Mann-Whitney *U* test. The overall survival rate and the cumulative recurrence rate after surgical resection were calculated using the Kaplan–Meier method. IBM SPSS Statistics (version 24) software was used for these analyses. *P* values less than 0.05 were considered significant.

2.5. Ethics

This study was approved by the Ethics Committees of the Keio University School of Medicine (ID: 20040034) and the National Cancer Center (ID: 2007–022), Tokyo, Japan, and was conducted in accordance with the principles of the Declaration of Helsinki.

3. Results

3.1. Evaluation of areas containing fibrous stroma within HCC tumors and their characteristics

The percentages of the tumor area containing fibrous stroma for each nodule are shown in Fig. 2A. Cases were divided into two groups according to the percentage of the tumor area containing fibrous stroma: 37 cases had ≥50% tumor area with fibrous stroma and 83 cases had <50% of the tumor area with fibrous stroma. Of the 37 cases with ≥50% tumor area

Table 2 Univariate analysis of clinicopathological parameters between nonsteatotic and steatotic sHCC

		Nonsteatotic sHCC (n = 16)		Steatotic sHCC (n = 21)		χ^2 Test *P*
Age	≥67 y [a]	7	43.8%	17	81.0%	.019
	<67 y	9	56.2%	4	19.0%	
Sex	Male	15	93.8%	16	76.2%	.206
	Female	1	6.2%	5	23.8%	
Tumor size	≥3.0 cm [b]	5	31.3%	9	42.9%	.471
	<3.0 cm	11	68.7%	12	57.1%	
Hepatitis B virus	Positive	6	37.5%	1	4.8%	.029 [c]
	Negative	10	62.5%	20	95.2%	
Hepatitis C virus	Positive	5	31.3%	4	19.0%	.458 [c]
	Negative	11	68.7%	17	81.0%	
Non-B, non-C	Non-B, non-C	5	31.3%	16	76.2%	.006
	Others	11	68.7%	5	23.8%	
Background liver inflammation	Normal liver, chronic hepatitis	11	68.7%	15	71.4%	1.000 [c]
	Precirrhosis, liver cirrhosis	5	31.3%	6	28.6%	
Tumor grade	Well differentiated	1	6.3%	3	14.3%	.395
	Moderately differentiated	14	87.4%	18	85.7%	
	Poorly differentiated	1	6.3%	0	0.0%	
Portal vein invasion and/or intrahepatic metastasis	Positive	11	68.7%	9	42.9%	.117
	Negative	5	31.3%	12	57.1%	
Venous invasion	Positive	0	0.0%	2	9.5%	.495 [c]
	Negative	16	100.0%	19	90.5%	
Bile duct invasion	Positive	1	6.2%	0	0.0%	.432 [c]
	Negative	15	93.8%	21	100.0%	
Tumor staging	Stage I	7	43.8%	12	57.1%	.419
(TNM)	Stage II	9	56.2%	9	42.9%	
Mallory-Denk bodies	+	9	56.2%	12	57.1%	.957
	−	7	43.8%	9	42.9%	
Tumor cell ballooning	+	9	56.2%	20	95.2%	.012 [c]
	−	7	43.8%	1	4.8%	
Inflammatory cell infiltration	+	13	81.2%	20	95.2%	.296 [c]
	−	3	18.8%	1	4.8%	
Steatohepatitic features [d]	+	7	43.8%	20	95.2%	.001 [c]
	−	9	56.3%	1	4.8%	
Background liver steatosis	≥5%	12	75.0%	17	81.0%	.705 [c]
	<5%	4	25.0%	4	19.0%	
Alcohol intake [e]	+	1	6.2%	2	9.5%	1.000 [c]
	−	15	93.8%	19	90.5%	
NASH	NASH	0	0.0%	5	23.8%	.057 [c]
	others	16	100.0%	16	76.2%	
ASH	ASH	0	0.0%	2	9.5%	.495
	others	16	100.0%	19	90.5%	
Diabetes mellitus	+	2	12.5%	10	47.6%	.024
	−	14	87.5%	11	52.4%	

Abbreviations: sHCC, scirrhous hepatocellular carcinoma; NASH, nonalcoholic steatohepatitis; ASH, alcoholic steatohepatitis.

[a] Average age of steatotic and nonsteatotic sHCC patients was 67 years.

[b] Average tumor size for steatotic and nonsteatotic sHCC cases was 3.0 cm. The range for nonsteatotic sHCC was 1.4–6.5 cm, and the range for steatotic sHCC was 0.7–7.0 cm.

[c] Using Fisher's direct method.

[d] sHCC fulfilling three of the following four criteria: steatosis, Mallory-Denk bodies, tumor cell ballooning, and inflammatory cell infiltration.

[e] Intake of ≥60 g (male) or ≥40 g (female) alcohol per day.

with fibrous stroma, 31 (83.8%) had more than 80% tumor area with fibrous stroma.

The macroscopic features and high-power views of the two groups are summarized in Fig. 2B. HCC with ≥50% tumor area with fibrous stroma had scalloped and irregular borders, the cut surface did not bulge, the growth pattern was infiltrative, and there was no fibrous capsule. In contrast, HCC with <50% of tumor area with fibrous stroma had round and regular borders, a bulging cut surface, expansive growth patterns, and a fibrous capsule. Based on these findings, we defined cases

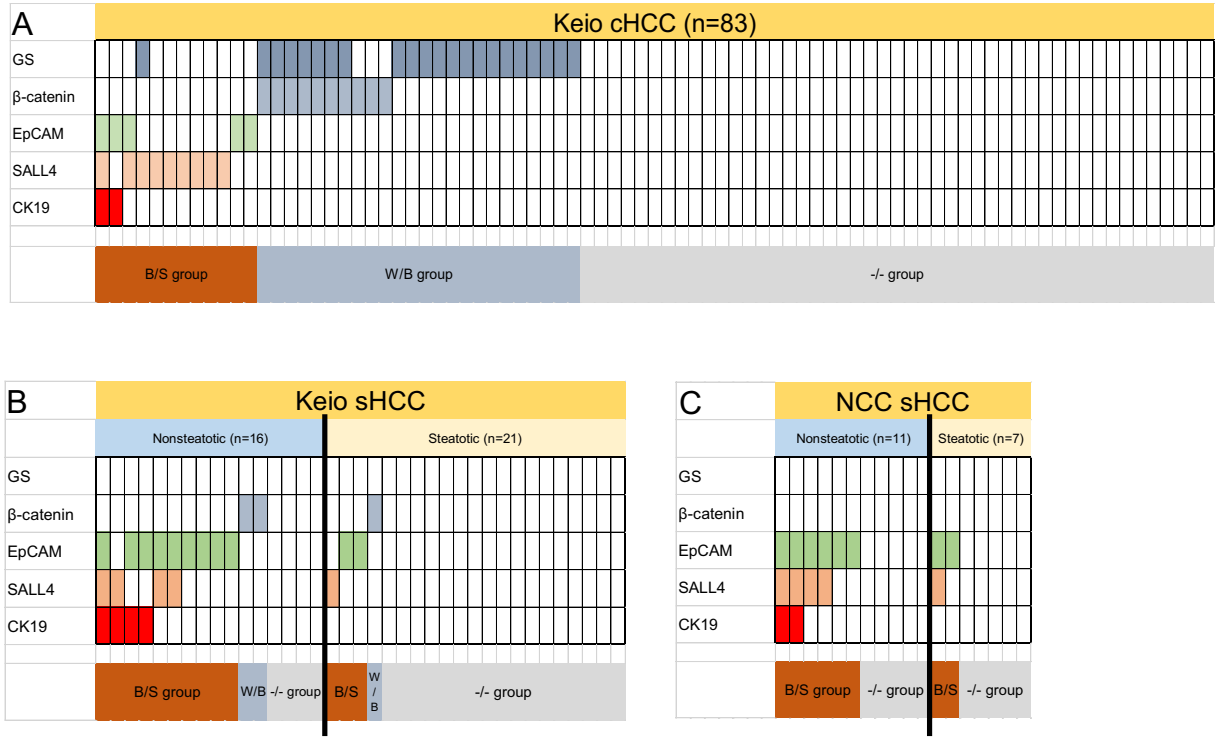


Fig. 4 Heatmap of immunohistochemical findings for the 120 sHCC and cHCC cases from Keio University Hospital and the 18 sHCC cases of the National Cancer Center validation set. The five immunohistochemical markers investigated are listed in the leftmost columns. Positive staining is shown as colored boxes and negative staining is shown in white. A, Keio cHCC (n = 83). B, Keio sHCC (n = 37). C, NCC sHCC (n = 18).

containing ≥50% tumor area with fibrous stroma as scirrhous HCC (sHCC), and those with <50% of tumor area of fibrous stroma as common HCC (cHCC). When sHCC was defined using a cut-off value of ≥80% tumor area with fibrous stroma, some cases which macroscopically showed features of sHCC were histologically classified as cHCC.

Samples from all 120 patients underwent epithelial membrane antigen staining and Alcian blue–periodic acid Schiff staining. The findings confirmed that none of the patients' tumors had features of combined hepatocellular–cholangiocarcinoma.

3.2. Correlation between HCC type and clinicopathological parameters

Correlations between the HCC type and clinicopathological parameters were examined (Table 1). sHCC had a

Table 3 Immunohistochemical features of sHCC according to the proportion of steatotic cells

	sHCC (n = 37)		sHCC (validation set from NCC, n = 18)		cHCC (n = 83)
	Nonsteatotic	Steatotic	Nonsteatotic	Steatotic	
B/S	10	3	6	2	12
	62.5%	14.3%	54.5%	28.6%	14.5%
W/B	2	1	0	0	24
	12.5%	4.7%	0.0%	0.0%	28.9%
–/–	4	17	5	5	47
	25.0%	81.0%	45.5%	71.4%	56.6%
Total	16	21	11	7	83
	100.0%	100.0%	100.0%	100.0%	100.0%
	$P = .002$ (Fisher's direct method)		$P = .367$ (Fisher's direct method)		

Abbreviations: sHCC, scirrhous hepatocellular carcinoma; NCC, National Cancer Center; cHCC: common hepatocellular carcinoma; B/S, the biliary/stem cell marker-positive group; W/B, the Wnt/β-catenin signaling–related markers–positive group; –/–, negative group.

Table 4 Univariate analysis of clinicopathological parameters associated with nonsteatotic and steatotic sHCC in the NCC validation set

		Nonsteatotic sHCC (n = 11)		Steatotic sHCC (n = 7)		P [c]
Age	≥63 years [a]	6	54.5%	4	57.1%	1.000
	<63 years	5	45.5%	3	42.9%	
Sex	Male	9	81.8%	7	100.0%	.231
	Female	2	18.2%	0	0.0%	
Tumor size	≥5.6 cm [b]	5	45.5%	3	42.9%	1.000
	<5.6 cm	6	54.5%	4	57.1%	
Hepatitis B virus	Positive	6	54.5%	1	14.3%	.151
	Negative	5	45.5%	6	85.7%	
Hepatitis C virus	Positive	1	9.1%	0	0.0%	1.000
	Negative	10	90.9%	7	100.0%	
Non-B, non-C	Non-B, non-C	4	36.4%	6	85.7%	.066
	others	7	63.6%	1	14.3%	
Background liver inflammation	Normal liver, chronic hepatitis	8	72.7%	5	71.4%	1.000
	Precirrhosis, liver cirrhosis	3	27.3%	2	28.6%	
Tumor grade	Well differentiated	0	0.0%	0	0.0%	1.000
	Moderately differentiated	9	81.8%	5	71.4%	
	Poorly differentiated	2	18.2%	2	28.6%	
Portal vein invasion and/or intrahepatic metastasis	Positive	5	45.5%	5	71.4%	.367
	Negative	6	54.5%	2	28.6%	
Venous invasion	Positive	2	18.2%	1	14.3%	1.000
	Negative	9	81.8%	6	85.7%	
Bile duct invasion	Positive	5	45.5%	2	28.6%	.637
	Negative	6	54.5%	5	71.4%	
Tumor staging (TNM)	Stage I	4	36.4%	2	28.6%	1.000
	Stage II	7	63.6%	5	71.4%	
Mallory-Denk bodies	+	2	18.2%	1	14.3%	1.000
	−	9	81.8%	6	85.7%	
Tumor cell ballooning	+	4	36.4%	2	28.6%	1.000
	−	7	63.6%	5	71.4%	
Inflammatory cell infiltration	+	11	100.0%	7	100.0%	1.000
	−	0	0.0%	0	0.0%	
Steatohepatitic features [d]	+	2	18.2%	2	28.6%	1.000
	−	9	81.8%	5	71.4%	
Background liver steatosis	≥5%	6	54.5%	3	42.9%	1.000
	<5%	5	45.5%	4	57.1%	

Abbreviations: sHCC, scirrhous hepatocellular carcinoma; NCC, National Cancer Center.

[a] Average age of steatotic and nonsteatotic sHCC patients in the NCC validation set was 63 years.

[b] Average tumor size for steatotic and nonsteatotic sHCC cases in the NCC validation set was 5.6 cm. The range for nonsteatotic sHCC was 2.5–9.5 cm, and the range for steatotic sHCC was 2.0–12.0 cm.

[c] Using Fisher's direct method.

[d] sHCC fulfilling three of the following four criteria: steatosis, Mallory-Denk bodies, tumor cell ballooning, and inflammatory cell infiltration.

significantly smaller tumor size (P = .012), fewer poorly differentiated tumors (P = .037), more tumor cell steatosis (P = .025), more Mallory-Denk bodies (P = .002), more tumor cell ballooning (P = .003), more inflammatory cell infiltration (P < .001), more steatohepatitic features (P < .001), more background liver steatosis (P = .030), and more NASH patients (P = .028) than cHCC did. The other factors had no significant correlations. The percentage of steatotic cells within each tumor was investigated and the results are plotted in Fig. 3A. The distribution of steatosis in sHCC was significantly different from that of cHCC (median percentage of tumor cells with steatosis: 10% and 2% for sHCC and cHCC, respectively, P = .009). When a cut-off value of ≥80% of the tumor area with fibrous stroma was used to define sHCC, no significant correlations were seen in terms of background liver steatosis or NASH patients (Supplementary Table 4). Based on these results, a cut-off value of 50% was used for further evaluation.

3.3. Clinicopathological features of sHCC according to the percentage of steatotic cells

sHCC cases had a wider distribution of the percentage of steatotic tumor cells than cHCC cases did (Fig. 3A). Steatotic HCC is commonly defined as tumors with ≥5% steatotic cells.

Steatosis of background liver is defined as the presence of lipid droplets in ≥5% of hepatocytes, and we used the same criterion for evaluating tumor cell steatosis: steatotic sHCC contains ≥5% steatotic hepatocytes and nonsteatotic sHCC contains <5% steatotic hepatocytes [10]. Among the 37 sHCC cases in this study, 21 (56.8%) were steatotic and 16 (43.2%) were nonsteatotic (Table 1). The clinicopathological features of steatotic and nonsteatotic sHCC are shown in Table 2. Compared with steatotic sHCC, nonsteatotic sHCC patients were younger (P = .019), had a higher rate of HBV (P = .029), a lower rate of non-B, non-C (P = .006), less tumor cell ballooning (P = .012), fewer steatohepatitic features (P = .001), and fewer cases of diabetes mellitus (P = .024). Although not significant, more nonsteatotic sHCC patients than steatotic sHCC patients tended to have vp/im (P = .117), and no nonsteatotic sHCC was seen in NASH patients (P = .057). Within non-B, non-C cases, the background liver inflammation and fibrosis status of nonsteatotic sHCC patients (n = 5) were normal liver 2 (40.0%), chronic hepatitis 2 (40.0%), and liver cirrhosis 1 (20.0%). For steatotic sHCC patients (n = 16), the numbers were normal liver 3 (18.7%), chronic hepatitis 9 (56.3%), precirrhosis 1 (6.3%), and liver cirrhosis 3 (18.7%).

No significant difference was seen in cumulative recurrence rates (P = .702), but there was a trend for steatotic sHCC patients to have a longer time to recurrence than nonsteatotic sHCC and cHCC patients (Fig. 3B).

3.4. Immunohistochemical subclassification of sHCC

The numbers of nonsteatotic sHCC patients (n = 16) with positive expression of CK19, SALL4, EpCAM, β-catenin, and GS were 4 (25.0%), 4 (25.0%), 9 (56.3%), 2 (12.5%), and 0 (0.0%), respectively, and for steatotic sHCC patients (n = 21), the numbers were 0 (0.0%), 1 (4.8%), 2 (9.5%), 1 (4.8%), and 0 (0.0%), respectively. Positive expressions in cHCC patients (n = 83) were 2 (2.4%), 9 (10.8%), 5 (6.0%), 10 (12.0%), and 22 (26.5%), respectively (Fig. 4). In total, 62.5% (n = 10) of nonsteatotic sHCC cases were found to be in the B/S group, whereas only 14.3% (n = 3) and 14.5% (n = 12) of steatotic sHCC and cHCC patients, respectively, were in the B/S group. Most steatotic sHCC patients (17 [81.0%]) and about half of cHCC patients (47 [56.6%]) were categorized in the −/− group, but only 4 (25.0%) nonsteatotic sHCC patients were in the −/− group, a much lower percentage. Only 2 (12.5%) nonsteatotic sHCC patients and 1 (4.7%) steatotic sHCC patient were in the W/B group, whereas 24 (28.9%) cHCC patients were in the W/B group (Table 3).

3.5. Validation sHCC study

Eighteen sHCC cases were selected from the database of the National Cancer Center, Tokyo, according to our criteria and the corresponding tissue samples were immunohistochemically analyzed as a validation study (Table 3). Of these 18 sHCC cases, 11 were categorized as nonsteatotic and 7 were categorized as steatotic. In total, 6 of 11 (54.5%) nonsteatotic sHCC cases were in the B/S group, and 5 of 7 (71.4%) steatotic sHCC cases were in the −/− group (P = .367). None of the 18 sHCC cases were in the W/B group. A higher rate of HBV was seen in nonsteatotic sHCC patients (6 [54.5%]) compared to steatotic sHCC patients (1 [14.3%]) (P = .151), and the frequency of non-B, non-C patients was higher in steatotic sHCC patients (6 [85.7%]) compared to nonsteatotic sHCC patients (4 [36.4%]) (P = .066) (Table 4). Although these differences were not statistically significant, analysis of a larger sample of validation cases could reveal these differences to be significant.

4. Discussion

There is currently insufficient data to define scirrhous HCC as an independent entity. Many definitions, clinicopathological features, and prognoses for scirrhous HCC have been described in previous reports ([13-19], Supplementary Table 3). Our findings should help clarify the characteristics and uniqueness of scirrhous HCC. Initially, we quantitatively evaluated the proportion of the tumor area containing fibrous stroma. Various cut-off values, ranging from 25% to 50%, have been reported for the percentage of tumor area with fibrous stroma for defining scirrhous HCC. According to our results in Fig. 2A, the downward-slope of the bar graph become steep in two places: one at around 80% and the other at around 50%. Therefore, cut-off values of both 50% and 80% were considered. When cut-offed at 80%, some cases that were histologically classified in the cHCC group had macroscopic features of sHCC. In addition, with an 80% cut-off, no significant correlations were seen in two clinicopathological factors (background liver steatosis and NASH patients) that were significant when using a cut-off of 50% (Table 1). A cut-off value of 50% also conformed with that in many previous reports [13,14,18,19]. For these reasons, we carried out the study using a 50% cut-off value. We have added the results of univariate analysis with an 80% cut-off value as Supplementary Tables 4 and 5. In the current study, we named the group of scirrhous tumors as “sHCC” to avoid confusion with previous descriptions of scirrhous HCC. There is a deviation in the prevalence of sHCC in our cohort (37/120 cases, 31%) and the prevalence of scirrhous HCC in the literature (5%) [1]. The reason for this may be that many cHCC patients are treated with radiofrequency ablation (RFA) or transarterial chemoembolization (TACE) prior to surgical excision, and so would have been excluded from the current study. In contrast, because of its atypical border in radiographic images, sHCC is often surgically excised without previous treatment with RFA or TACE. The high prevalence of sHCC in the current study likely reflects this selection bias.

Interestingly, by examining sHCC in detail microscopically, we found two histological subgroups within sHCC: sHCC with steatosis (steatotic sHCC) and sHCC without

steatosis (nonsteatotic sHCC). This further classification of sHCC enabled us to clearly identify different etiological backgrounds and molecular features, indicating different carcinogenic pathways for steatotic sHCC, nonsteatotic sHCC, and cHCC. Our results showed high rates of HBV in nonsteatotic sHCC and high frequencies of non-B, non-C patients in steatotic sHCC; both were statistically significant. In general, the hepatitis virus infection rate and the virus type depend on the country or region to which the cohort belongs. For example, HCV infection rates are high in Japan, whereas HBV infection rates are high in Korea. In previous reports on scirrhous HCC, because the presence of steatosis in the tumor was not considered, viral infection rates varied and depended heavily on the cohort studied. Therefore, each study tended to identify different "unique characteristics" for scirrhous HCC in comparison with classical HCC. This finding, that steatotic and nonsteatotic sHCC have different characteristics, may well help explain why scirrhous HCC has not yet been clearly defined as an independent entity.

Compared with steatotic sHCC and cHCC patients, nonsteatotic sHCC patients had a trend of high HBV rates, an increased frequency of vp/im, a shorter time to recurrence, and an increased frequency of positive expression of biliary/stem cell markers. The up-regulation of epithelial–mesenchymal transition (EMT)-related proteins is seen in CK19-positive HCCs [20], and EMT leads to cancer invasion and metastasis [19]. We previously reported that HCCs with positive expression of CK19, SALL4, and EpCAM show aggressive behavior [9]. Taken together, the evidence suggests that nonsteatotic sHCC exhibits aggressive biological behavior. Moreover, in Korea, which has a background of high HBV infection rates, scirrhous HCC is reportedly associated with high HBV rates, the expression of stem cell markers and EMT-related genes, overexpression of transforming growth factor-β signaling proteins, and significantly shorter disease-free survival [19]. Another study from Korea reported that CK19 and EpCAM-positive HCC with HBV infection is correlated with alpha-smooth muscle actin and cancer-associated fibroblast accumulation [21]. Our nonsteatotic sHCC has many characteristics in common with these scirrhous HCC cases reported in Korea, and they may be overlapping entities. We consider that HCCs associated with HBV infection and EMT, such as our nonsteatotic sHCC cases, constitute a major subgroup of scirrhous HCC.

Compared to nonsteatotic sHCC and cHCC, steatotic sHCC, in addition to the unique histopathological characteristics of tumor cell steatosis and fibrous stroma, commonly had a non-B, non-C etiological background and often belonged to the immunohistochemically negative (−/−) group. Recently, a variant of HCC named steatohepatitic HCC (SH-HCC) was proposed [12,22,23]. SH-HCC features tumor cell steatosis occurring against a non-B, non-C background and appears to be related to NASH/NAFLD and diabetes mellitus. Low nuclear accumulation of β-catenin and low expression levels of GS, SALL4, and EpCAM have been reported in SH-HCC [24]. Moreover, SH-HCC patients have a relatively good prognosis [12]. These findings indicate that SH-HCC is similar to our steatotic sHCC and support the suggestion that steatotic sHCC exhibits less aggressive biological behavior. The criteria defining SH-HCC do not completely overlap those for steatotic sHCC, and the definition of SH-HCC is still controversial. For example, HCC with tumor cell steatosis, Mallory-Denk bodies, tumor cell ballooning or intertumoral inflammatory cell infiltration could be diagnosed as SH-HCC; fibrous stroma is not a necessary criterion for SH-HCC, according to Salomao et al [22,23] Further investigations are needed to elucidate the differences between SH-HCC and steatotic sHCC.

In the current study, the frequencies of both nonsteatotic and steatotic sHCC cases immunohistochemically subclassified as W/B were significantly low compared to that of cHCC. A 2007 report by Audard et al indicated that HCC with strong GS expression correlates with histologically microtrabecular and acinar patterns with cholestasis and rare steatosis [25]. A recent report by Calderaro et al showed that HCC with β-catenin nuclear staining and strong GS expression also has a microtrabecular pattern with cholestasis. These tumors were categorized in molecular classifications G5 and G6, indicating good differentiation [26]. The types of HCC described by Audard et al and Calderaro et al show the histological features of our cHCC. Although Wnt/β-catenin pathway activation is typical in the carcinogenesis of HCC [27,28], the activation of pathways involving β-catenin is considered to be rare in sHCC because the number of sHCC cases in the W/B group was significantly low. Detailed analyses of gene expression and pathway activation in sHCC cases are left to further studies.

5. Conclusion

We elucidated the unique histopathological characteristics of sHCC by first making detailed quantitative evaluations of the tumor area containing fibrous stroma. By performing stratified classification of sHCC cases with or without steatosis, we could clarify and compare the etiological background, molecular pathological features, and biological behaviors of sHCC. We consider sHCC to be an entity distinct from cHCC, and this may prove to be an important classification for informing the choice of clinical treatment.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.11.024.

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References

[1] Theise ND, Curado MP, Franceschi S, et al. Hepatocellular carcinoma. In: Bosman E, Carneiro F, Hruban RH, Theise ND, editors. WHO Classification of Tumors of the Digestive System. 4th ed. Lyon, France: International Agency for Research on Cancer (IARC); 2010. p. 205-16.

[2] Omata M, Peters RL, Tatter D. Sclerosing hepatic carcinoma: relationship to hypercalcemia. Liver 1981;1:33-49.

[3] Albar JP, De Miguel F, Esbrit P, Miranda R, Fernandes-Flores A, Sarasa JL. Immunohistochemical detection of parathyroid hormone-related protein in a rare variant of hepatic neoplasm (sclerosing hepatic carcinoma). Hum Pathol 1996;27:728-31.

[4] Kitazawa R, Kitazawa S, Yoon S, Kasuga M, Maeda S. In situ demonstration of parathyroid hormone-related protein mRNA in sclerosing hepatic carcinoma. Virchows Arch 1999;435:137-42.

[5] Yamamoto M, Ariizumi S, Yoshitoshi K, Saito A, Nakano M, Takasaki K. Hepatocellular carcinoma with a central scar and a scalloped tumor margin resembling focal nodular hyperplasia in macroscopic appearance. J Surg Oncol 2006;94:587-91.

[6] Hoshida Y, Toffanin S, Lachenmayer A, Villanueva A, Minguez B, Llovet JM. Molecular classification and novel targets in hepatocellular carcinoma: recent advancements. Semin Liver Dis 2010;30:35-51.

[7] Boyault S, Rickman DS, de Reynies A, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology 2007;45:42-52.

[8] Zucman-Rossi J, Villanueva A, Nault JC, Llovet JM. Genetic landscape and biomarkers of hepatocellular carcinoma. Gastroenterology 2015;149:1226-1239.e4.

[9] Tsujikawa H, Masugi Y, Yamazaki K, Itano O, Kitagawa Y, Sakamoto M. Immunohistochemical molecular analysis indicates hepatocellular carcinoma subgroups that reflect tumor aggressiveness. Hum Pathol 2016;50:24-33.

[10] Sakamoto M, Tsujikawa H, Effendi K, et al. Pathological findings of nonalcoholic steatohepatitis and nonalcoholic fatty liver disease. Pathol Int 2017;67:1-7.

[11] Bedossa P, Poitou C, Veyrie N, et al. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. Hepatology 2012;56:1751-9.

[12] Shibahara J, Ando S, Sakamoto Y, Kokudo N, Fukayama M. Hepatocellular carcinoma with steatohepatitic features: a clinicopathological study of Japanese patients. Histopathology 2014;64:951-62.

[13] Matsuura S, Aishima S, Taguchi K, et al. 'Scirrhous' type hepatocellular carcinomas: a special reference to expression of cytokeratin 7 and hepatocyte paraffin 1. Histopathology 2005;47:382-90.

[14] Okamura N, Yoshida M, Shibuya A, Sugiura H, Okayasu I, Ohbu M. Cellular and stromal characteristics in the scirrhous hepatocellular carcinoma: comparison with hepatocellular carcinomas and intrahepatic cholangiocarcinomas. Pathol Int 2005;55:724-31.

[15] Kurogi M, Nakashima O, Miyaaki H, Fujimoto M, Kojiro M. Clinicopathological study of scirrhous hepatocellular carcinoma. J Gastroenterol Hepatol 2006;21:1470-7.

[16] Fujii T, Zen Y, Harada K, et al. Participation of liver cancer stem/progenitor cells in tumorigenesis of scirrhous hepatocellular carcinoma—human and cell culture study. Hum Pathol 2008;39:1185-96.

[17] Kim SH, Lim HK, Lee WJ, Choi D, Park CK. Scirrhous hepatocellular carcinoma: comparison with usual hepatocellular carcinoma based on CT-pathologic features and long-term results after curative resection. Eur J Radiol 2009;69:123-30.

[18] Lee JH, Choi MS, Gwak GY, et al. Clinicopathologic characteristics and long-term prognosis of scirrhous hepatocellular carcinoma. Dig Dis Sci 2012;57:1698-707.

[19] Seok JY, Na DC, Woo HG, et al. A fibrous stromal component in hepatocellular carcinoma reveals a cholangiocarcinoma-like gene expression trait and epithelial-mesenchymal transition. Hepatology 2012;55:1776-86.

[20] Kim H, Choi GH, Na DC, et al. Human hepatocellular carcinomas with "Stemness"-related marker expression: keratin 19 expression and a poor prognosis. Hepatology 2011;54:1707-17.

[21] Yoo JE, Kim YJ, Rhee H, et al. Progressive enrichment of stemness features and tumor stromal alterations in multistep hepatocarcinogenesis. PLoS One 2017;12:e0170465.

[22] Salomao M, Yu WM, Brown Jr RS, Emond JC, Lefkowitch JH. Steatohepatitic hepatocellular carcinoma (SH-HCC): a distinctive histological variant of HCC in hepatitis C virus-related cirrhosis with associated NAFLD/NASH. Am J Surg Pathol 2010;34:1630-6.

[23] Salomao M, Remotti H, Vaughan R, Siegel AB, Lefkowitch JH, Moreira RK. The steatohepatitic variant of hepatocellular carcinoma and its association with underlying steatohepatitis. Hum Pathol 2012;43:737-46.

[24] Ando S, Shibahara J, Hayashi A, Fukayama M. Beta-catenin alteration is rare in hepatocellular carcinoma with steatohepatitic features: immunohistochemical and mutational study. Virchows Arch 2015;467:535-42.

[25] Audard V, Grimber G, Elie C, et al. Cholestasis is a marker for hepatocellular carcinomas displaying beta-catenin mutations. J Pathol 2007;212:345-52.

[26] Calderaro J, Couchy G, Imbeaud S, et al. Histological subtypes of hepatocellular carcinoma are related to gene mutations and molecular tumour classification. J Hepatol 2017;67:727-38.

[27] de La Coste A, Romagnolo B, Billuart P, et al. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. Proc Natl Acad Sci U S A 1998;95:8847-51.

[28] Miyoshi Y, Iwao K, Nagasawa Y, et al. Activation of the beta-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. Cancer Res 1998;58:2524-7.