Supplementary Material:

Sarcomatoid Hepatocellular Carcinoma is Distinct from Ordinary Hepatocellular Carcinoma: Clinicopathologic, Transcriptomic, and Immunologic Analyses

Ryo Morisue, Motohiro Kojima, Toshihiro Suzuki, Tetsuya Nakatsura, Hidenori Ojima, Reiko Watanabe, Motokazu Sugimoto, Shin Kobayashi, Shinichiro Takahashi, Masaru Konishi, Genichiro Ishii, Naoto Gotohda, Toshiyoshi Fujiwara, and Atsushi Ochiai

Table of contents

Materials and Methods

Transcriptome Analysis

After RNA-sequencing, the reads were aligned using bowtie2-2.2.9 to remove rRNA-derived reads and mapped to the human reference genome hg38 with Tophat-2.1.1. To generate a transcriptome assembly, the alignment-reads were inserted into Cufflinks packages (Cufflinks-2.2.1), and the expression level of each gene was calculated and normalized as fragments per kilobase of exon per million reads mapped values. The expression levels of each gene in OHCC and SHCC samples were illustrated in a heat map using TIBCO Spotfire™ Analyst 7.11.1 software (TIBCO, Palo Alto, California, USA), and hierarchical clustering was calculated by Ward's method. The principal component analysis was calculated by R software (version 4.0.0 for Windows; the R Foundation for Statistical Computing, Vienna, Austria). The sequencing coverage and quality statistics of each sample were summarized in Supplementary Table S1.

Fluorescent Multiplex Immunohistochemistry (FMIHC)

FMIHC was performed by the tyramide signal amplification (TSA™) method using an Opal™ IHC kit (PerkinElmer, Waltham, Massachusetts, USA) according to the manufacturer's instructions. When scanning the stained slides, fluorescent-labeled multiplexed images (669/500 μm each) of the tumor margin (10 fields or more) and center (10 fields or more) were separately captured with an automated imaging system (Vectra™ ver. 3.0, PerkinElmer). To assess SHCCs, each sarcomatous and carcinomatous component confirmed in bright field were imaged. inForm™ imaging analysis software (PerkinElmer) was used to segment each image into cancer cell nests (epithelial region) and frameworks (stromal region) and detect immune cells with specific phenotypes and components. Tissue segmentation and phenotype recognition were repeated until the algorithm reached the level of confidence recommended by the program supplier (at least 90% accuracy) before performing the evaluation. Infiltrating immune cells were quantified using an analytic software program (TIBCO) and then calculated per area. Using Spotfire, the $CD3^+$ population in $CD4^+$ and $CD8^+$ cells, programmed death-1^+ (PD-1^+) subset in T cells, and

programmed death-ligand 1^+ ($PD\text{-}L1^+$) subset in tumor cells were divided according to the fluorescence signal intensities of CD3, PD-1, and PD-L1, respectively. The level of PD-1 expression on each immune cell was calculated by the intensity of fluorescent PD-1 expression and normalized. Principal component analysis in the current study distributed samples into the three-dimensional spaces based on variances of 13 variables: the density of intratumoral $CD4^+/CD8^+/PD\text{-}1^+CD4^+/PD\text{-}1^+CD8^+$ T cells, stromal $CD4^+/CD8^+/PD\text{-}1^+CD4^+/PD\text{-}1^+CD8^+$ T cells, the level of PD-1 expression on intratumoral and stromal $CD4^+/CD8^+$ T cells, and the proportion of tumor cells with PD-L1 expression.

Table S2. Antibodies used in the current study

For fluorescent multiplexed immunohistochemistry					
Antigen	Clone	Isotype	Manufacturer	Retrieval condition	Dilution
CD3	SP7	Rabbit IgG	Abcam	TRS9 (Dako), 95°C, 15 minutes	1:600
CD4	4B12	Mouse IgG1	Novocastra	TRS9 (Dako), 95°C, 15 minutes	1:200
CD8	4B11	Mouse IgG2a	Novocastra	TRS9 (Dako), 95°C, 15 minutes	1:160
PD-1	EH33	Mouse IgG2a	Cell Signaling Technology	TRS9 (Dako), 95°C, 15 minutes	1:200
PD-L1	E1L3N	Rabbit IgG	Cell Signaling Technology	TRS9 (Dako), 95°C, 15 minutes	1:1200
For immunohistochemical assays					
Antigen	Clone	Isotype	Manufacturer	Retrieval condition	Dilution
HepPar-1	OCH1E5	Mouse IgG1, kappa	Dako/M7158	CC1 (Roche), 95°C, 36 minutes	1:100
Vimentin	Vim3B4	Mouse IgG2a, kappa	Dako	CC1 (Roche), 95°C, 20 minutes	1:500
AE1/AE3	AE1, AE3, PCK26	Mouse IgG1	Roche (VENTANA)	Protease1, 4 minutes	Ready to use
PD-L1	E1L3N	Rabbit IgG	Cell Signaling Technology	CC2 (Roche), 100°C, 56 minutes	1:200
PD-L1	SP263	Rabbit IgG	Roche (VENTANA)	CC1 (Roche), 100°C, 64 minutes	Ready to use
MLH1	M1	Mouse IgG	Roche (VENTANA)	CC1 (Roche), 100°C, 64 minutes	Ready to use
MSH2	G219-1129	Mouse IgG1	Roche (VENTANA)	CC1 (Roche), 100°C, 64 minutes	Ready to use
MSH6	SP93	Rabbit IgG	Roche (VENTANA)	CC1 (Roche), 100°C, 64 minutes	Ready to use
PMS2	A16-4	Mouse IgG1	Roche (VENTANA)	CC1 (Roche), 100°C, 64 minutes	Ready to use
ARID1A	JJ09-01	Rabbit IgG	NOVUS BIOLOGICALS	CC1 (Roche), 95°C, 64 minutes	1:50
INI-1	MRQ-27	Mouse IgG2a	CELL MARQUE	CC1 (Roche), 95°C, 64 minutes	1:100
SMARCA2	BRM (ab15597)	Rabbit IgG	Abcam	CC1 (Roche), 95°C, 64 minutes	1:50
BRG1	EPNCIR111A	Rabbit IgG	Abcam	CC1 (Roche), 95°C, 64 minutes	1:200
p53	DO-7	Mouse IgG1, kappa	Roche (VENTANA)	CC1 (Roche), 95°C, 64 minutes	Ready to use
β-catenin	6B3	Rabbit IgG	Cell Signaling Technology	CC1 (Roche), 95°C, 64 minutes	1:200

Abbreviations: TRS9, Target retrieval solution, pH9.0, 10x; CC1, pH8.5 EDTA buffer; CC2, pH6.0 citrate buffer

Table S3.

Clinicopathological characteristics of patients with SHCC or OHCC in cohort A.

Variables	SHCC (n=14)	OHCC (n=163)	*p* value
Age, median (range), years	69 (54–76)	69 (36–89)	0.566
Male sex, n (%)	12 (85.7)	136 (83.4)	1.000
Risk factor for liver injury, n (%)			0.005
HCV infection	3 (21.4)	74 (45.4)	
HBV infection	2 (14.3)	31 (19.0)	
HCV and HBV coinfection	2 (14.3)	0 (0)	
Alcohol	2 (14.3)	24 (14.7)	
Undetermined	5 (35.7)	34 (20.9)	
Diabetes, n (%)	3 (21.4)	42 (25.8)	1.000
Prior treatment, n (%)	3 (21.4)	15 (9.2)	0.157
TACE	2 (14.3)	8 (4.9)	
RFA	1 (7.1)	4 (2.5)	
TACE and RFA	0 (0)	2 (1.2)	
TACE and Proton beam	0 (0)	1 (1.2)	
Curative intent, n (%)	13 (92.9)	154 (94.5)	0.571
Child-Pugh grade, A / B, n (%)	10 (71.4) / 4 (28.6)	157 (96.3) / 6 (3.7)	0.004
Cirrhosis, n (%)	4 (28.6)	42 (25.8)	0.760
AST, median (range), U/L	33 (16–144)	35 (11–206)	0.855
ALT, median (range), U/L	30 (10–112)	31 (7–272)	0.704
Total bilirubin, median (range), mg/dL	0.6 (0.4–1.5)	0.7 (0.3–2.0)	0.143
Albumin, median (range), g/dL	3.7 (2.7–4.6)	4.2 (2.5–5.1)	0.003
Platelet count, median (range), $\times 10^9$/L	171 (70–416)	152 (38–423)	0.466
Fibrosis-4 index, median (range)	3.63 (0.78–6.04)	2.93 (0.79–15.59)	0.972
ICGR15, median (range), %	11.7 (7.1–20.6)	12.4 (2.3–32.6)	0.925
CRP, median (range), mg/dL	0.60 (0.03–12.77)	0.10 (0.01–10.63)	0.010
Neutrophil count, median (range), $\times 10^9$/L	356 (136–880)	290 (83–763)	0.032
Lymphocyte count, median (range), $\times 10^9$/L	99 (34–187)	154 (45–420)	<0.001
NLR, median (range)	3.67 (1.61–12.57)	1.88 (0.56–8.12)	<0.001
AFP, median (range), ng/mL	14.7 (1.6–54812.5)	11.5 (1.5–177589)	0.517
PIVKA-II, median (range), mAU/mL	325 (15–12335)	93 (10–411420)	0.635
Tumor size, median (range), mm	53 (12–220)	35 (11–270)	0.084
Multiplicity, n (%)	3 (21.4)	42 (25.8)	1.000
Macrovascular invasion, n (%)	3 (21.4)	4 (2.5)	0.013
Microvascular invasion, n (%)	6 (42.9)	41 (25.9)	0.211
Surgical margin positive, n (%)	2 (14.3)	1 (0.6)	0.017

Continued

Grade*, n (%)			<0.001
1	0 (0)	14 (8.7)	
2	0 (0)	121 (75.2)	
3	14 (100)	26 (16.1)	
UICC stage, n (%)			0.027
I	3 (21.4)	91 (55.8)	
II	6 (42.9)	49 (30.1)	
III	4 (28.6)	19 (11.7)	
IV	1 (7.1)	4 (2.5)	
BCLC stage, n (%)			0.102
0	0 (0)	16 (9.8)	
A	3 (21.4)	71 (43.6)	
B	1 (7.1)	16 (9.8)	
C	10 (71.4)	60 (36.8)	
LCSGJ stage, n (%)			0.086
I	0 (0)	21 (12.9)	
II	4 (28.6)	79 (48.5)	
III	8 (57.1)	48 (29.4)	
IV	2 (14.3)	15 (9.2)	
Relapse, n (%)	13 (92.9)	114 (69.9)	0.117
Intrahepatic relapse, n (%)	10 (71.4)	102 (62.6)	0.578
Extrahepatic relapse, n (%)	6 (42.9)	15 (9.2)	0.002
Relapse site, n (%)			
Lymph node	4 (28.6)	4 (2.5)	0.001
Peritoneum	2 (14.3)	3 (1.8)	0.051
Lung	2 (14.3)	6 (3.7)	0.124
Bone	0 (0)	2 (1.2)	1.000
Relapse within 6 months, n (%)	7 (50.0)	24 (14.7)	0.004
Relapse within 1 year, n (%)	9 (64.3)	43 (26.4)	0.005

*Grade was assessed according to the 5th edition of WHO Classification of Digestive System Tumours.

Abbreviations: SHCC, sarcomatoid hepatocellular carcinoma; OHCC, ordinary hepatocellular carcinoma; HCV; hepatitis C virus; HBV, hepatitis B virus; TACE, transcatheter arterial chemoembolization; RFA, radiofrequency ablation; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ICGR15, indocyanine green retention rate at 15 minutes; CRP, C-reactive protein; NLR, neutrophil-to-lymphocyte; AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; UICC, Union for International Cancer Control; BCLC, Barcelona Clinic Liver Cancer; LCSGJ, Liver Cancer Study Group of Japan.

Table S4.

Cox proportional hazards regression models for overall survival, disease-specific survival, and relapse-free survival in cohort A (n=177).

Variables	Overall survival						Disease-specific survival						Relapse-free survival					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value
Age (≥65 vs. <65 years)	1.087	0.612–1.928	0.777				1.016	0.539–1.916	0.962				1.072	0.734–1.566	0.718			
Sex (male vs. female)	0.714	0.369–1.382	0.317				0.696	0.335–1.447	0.332				0.864	0.540–1.380	0.540			
Viral infection (yes vs. no)	0.752	0.441–1.282	0.294				0.799	0.440–1.452	0.462				1.291	0.892–1.870	0.176			
Cirrhosis (yes vs. no)	0.933	0.516–1.687	0.819				0.939	0.484–1.819	0.851				1.344	0.921–1.961	0.125			
Child-Pugh grade (B vs. A)	7.103	3.172–15.90	< 0.001	2.597	0.940–7.180	0.066	6.198	2.421–15.87	< 0.001	2.095	0.641–6.847	0.221	5.925	2.670–13.15	< 0.001	2.739	1.047–7.163	0.040
Tumor size (>50 vs. ≤50 mm)	2.641	1.560–4.470	< 0.001	1.111	0.591–2.089	0.743	2.533	1.409–4.553	0.002	1.054	0.522–2.129	0.884	1.520	1.058–2.183	0.023	0.960	0.620–1.488	0.856
Multiplicity (yes vs. no)	1.526	0.870–2.676	0.140				1.590	0.855–2.957	0.143				1.644	1.111–2.433	0.013	1.508	0.959–2.370	0.075
Microvascular invasion (yes vs. no)	3.118	1.840–5.283	< 0.001	2.933	1.613–5.334	< 0.001	3.133	1.740–5.640	< 0.001	3.099	1.574–6.102	0.001	2.242	1.540–3.263	< 0.001	2.149	1.401–3.297	< 0.001
Macrovascular invasion (yes vs. no)	6.683	2.619–17.05	< 0.001	4.702	1.401–15.78	0.012	9.371	3.621–24.25	< 0.001	5.980	1.629–21.95	0.007	3.188	1.395–7.286	0.006	2.152	0.707–6.548	0.177
Surgical margin (positive vs. negative)	4.266	1.027–17.73	0.046	1.972	0.406–9.589	0.400	4.266	1.027–17.73	0.046	2.103	0.420–10.53	0.366	25.50	7.084–91.80	< 0.001	12.82	2.872–57.25	0.001
NLR (>2.8 vs. ≤2.8)	3.062	1.757–5.337	< 0.001	1.591	0.784–3.227	0.198	2.934	1.582–5.444	0.001	1.506	0.672–3.375	0.320	1.700	1.124–2.569	0.012	1.225	0.741–2.025	0.429
UICC stage (III-IV vs. I-II)	5.088	2.903–8.920	< 0.001	1.883	0.828–4.280	0.131	5.426	2.923–10.07	< 0.001	1.769	0.704–4.447	0.225	3.464	2.187–5.489	< 0.001	1.519	0.733–3.147	0.261
Grade (3 vs. 1-2)	2.826	1.640–4.867	< 0.001	1.020	0.449–2.319	0.962	3.727	2.065–6.727	< 0.001	1.337	0.554–3.228	0.518	1.686	1.121–2.535	0.012	0.936	0.541–1.620	0.814
Group (SHCC vs. OHCC)	5.904	3.026–11.52	< 0.001	2.597	1.025–6.583	0.044	7.934	3.980–15.82	< 0.001	3.041	1.137–8.128	0.027	3.701	2.048–6.685	< 0.001	1.522	0.665–3.483	0.320

Abbreviations: NLR, neutrophil-to-lymphocyte ratio; UICC, Union for International Cancer Control; SHCC, sarcomatoid hepatocellular carcinoma; OHCC, ordinary hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval.

Table S5. Clinicopathological variables of patients for transcriptome analysis.

Case number	Age	Sex	Risk factor for liver injury	Grade	UICC stage	BCLC stage	LCSGJ stage
OHCC-1	61	Female	HCV	2	I	A	II
OHCC-2	69	Female	Undetermined	3	II	C	III
OHCC-3	80	Female	Undetermined	2	II	C	III
OHCC-4	76	Male	Undetermined	2	III	C	IV
OHCC-5	89	Female	HCV	3	I	A	II
SHCC-1	76	Male	Undetermined	3	II	C	III
SHCC-2	72	Female	Undetermined	3	IV	C	III

Abbreviation: UICC, Union for International Cancer Control; BCLC, Barcelona Clinic Liver Cancer; LCSGJ, Liver Cancer Study Group of Japan; HCV, hepatitis C virus

Table S6

Gene set enrichment analysis results of upregulated and downregulated hallmark gene sets (Molecular Signatures Database v7.1) in SHCC.

Gene sets upregulated in SHCC

NAME	MSigDB	SIZE	ES	NES	NOM *P*	FDR	FWER	RANK AT MAX
EPITHELIAL_MESENCHYMAL_TRANSITION	HALLMARK	185	0.74	1.75	0.00	0.00	0.00	2417
G2M_CHECKPOINT	HALLMARK	175	0.65	1.54	0.00	0.00	0.00	7328
MITOTIC_SPINDLE	HALLMARK	193	0.64	1.53	0.00	0.00	0.01	6109
MYC_TARGETS_V1	HALLMARK	175	0.62	1.48	0.00	0.00	0.01	6596
UV_RESPONSE_DN	HALLMARK	132	0.62	1.45	0.00	0.01	0.04	5747
HYPOXIA	HALLMARK	180	0.61	1.44	0.00	0.01	0.05	3619
DNA_REPAIR	HALLMARK	134	0.60	1.41	0.00	0.01	0.10	6504
APICAL_JUNCTION	HALLMARK	175	0.58	1.38	0.00	0.02	0.15	5766
TGF_BETA_SIGNALING	HALLMARK	52	0.63	1.37	0.02	0.02	0.17	5733
TNFA_SIGNALING_VIA_NFKB	HALLMARK	184	0.58	1.37	0.00	0.02	0.21	5373
E2F_TARGETS	HALLMARK	177	0.57	1.36	0.00	0.02	0.23	7185
WNT_BETA_CATENIN_SIGNALING	HALLMARK	39	0.61	1.32	0.04	0.03	0.41	6831
PROTEIN_SECRETION	HALLMARK	91	0.56	1.27	0.02	0.08	0.72	6086
MYC_TARGETS_V2	HALLMARK	53	0.57	1.25	0.07	0.11	0.86	6097
GLYCOLYSIS	HALLMARK	185	0.52	1.25	0.01	0.11	0.88	5663
UNFOLDED_PROTEIN_RESPONSE	HALLMARK	96	0.53	1.24	0.06	0.11	0.91	6557
INFLAMMATORY_RESPONSE	HALLMARK	192	0.52	1.23	0.02	0.12	0.92	5624
NOTCH_SIGNALING	HALLMARK	31	0.59	1.21	0.16	0.14	0.96	4838
IL2_STAT5_SIGNALING	HALLMARK	180	0.50	1.18	0.07	0.22	1.00	7950
PI3K_AKT_MTOR_SIGNALING	HALLMARK	95	0.50	1.17	0.15	0.24	1.00	7395
MYOGENESIS	HALLMARK	187	0.48	1.15	0.11	0.28	1.00	4861
HEDGEHOG_SIGNALING	HALLMARK	35	0.54	1.14	0.26	0.31	1.00	5094
ALLOGRAFT_REJECTION	HALLMARK	174	0.45	1.07	0.29	0.53	1.00	7095
APOPTOSIS	HALLMARK	144	0.45	1.06	0.33	0.55	1.00	5023
IL6_JAK_STAT3_SIGNALING	HALLMARK	81	0.46	1.06	0.39	0.56	1.00	5482
SPERMATOGENESIS	HALLMARK	130	0.45	1.05	0.36	0.54	1.00	6777
KRAS_SIGNALING_UP	HALLMARK	181	0.44	1.04	0.38	0.56	1.00	5154
ANGIOGENESIS	HALLMARK	32	0.50	1.03	0.45	0.60	1.00	2233
P53_PATHWAY	HALLMARK	175	0.43	1.03	0.44	0.59	1.00	6383
HEME_METABOLISM	HALLMARK	178	0.40	0.95	0.64	0.80	1.00	6380
APICAL_SURFACE	HALLMARK	41	0.44	0.94	0.62	0.81	1.00	5893
ESTROGEN_RESPONSE_EARLY	HALLMARK	183	0.38	0.90	0.80	0.89	1.00	5818
MTORC1_SIGNALING	HALLMARK	180	0.36	0.87	0.84	0.94	1.00	5674
UV_RESPONSE_UP	HALLMARK	142	0.34	0.80	0.91	1.00	1.00	5324
PANCREAS_BETA_CELLS	HALLMARK	38	0.36	0.78	0.83	1.00	1.00	3303
INTERFERON_GAMMA_RESPONSE	HALLMARK	169	0.33	0.78	0.94	0.98	1.00	6115
ANDROGEN_RESPONSE	HALLMARK	88	0.34	0.77	0.90	0.95	1.00	4799
ESTROGEN_RESPONSE_LATE	HALLMARK	180	0.32	0.76	0.97	0.94	1.00	5628

Gene sets downregulated in SHCC

NAME	MSigDB	SIZE	ES	NES	NOM P	FDR	FWER	RANK AT MAX
BILE_ACID_METABOLISM	HALLMARK	104	-0.78	-2.58	0.00	0.00	0.00	1496
XENOBIOTIC_METABOLISM	HALLMARK	181	-0.69	-2.50	0.00	0.00	0.00	1497
FATTY_ACID_METABOLISM	HALLMARK	145	-0.62	-2.18	0.00	0.00	0.00	1869
COAGULATION	HALLMARK	125	-0.52	-1.78	0.00	0.00	0.00	889
PEROXISOME	HALLMARK	91	-0.50	-1.59	0.00	0.01	0.01	804
CHOLESTEROL_HOMEOSTASIS	HALLMARK	66	-0.43	-1.39	0.03	0.04	0.03	1869
ADIPOGENESIS	HALLMARK	180	-0.34	-1.20	0.00	0.18	0.11	1731
COMPLEMENT	HALLMARK	170	-0.32	-1.19	0.00	0.16	0.12	1127
OXIDATIVE_PHOSPHORYLATION	HALLMARK	175	-0.32	-1.15	0.00	0.19	0.15	2308
REACTIVE_OXYGEN_SPECIES_PATHWAY	HALLMARK	44	-0.39	-1.13	0.15	0.18	0.16	1883
KRAS_SIGNALING_DN	HALLMARK	186	-0.28	-1.02	0.50	0.40	0.35	1922
INTERFERON_ALPHA_RESPONSE	HALLMARK	80	-0.30	-0.99	0.53	0.47	0.42	1258

Abbreviations: SHCC, sarcomatoid hepatocellular carcinoma; MSigDB, Molecular Signatures Database; ES, enrichment score; NES, normalized enrichment score; NOM P, nominal p value; FDR, false discovery rate; FWER, familywise-error rate

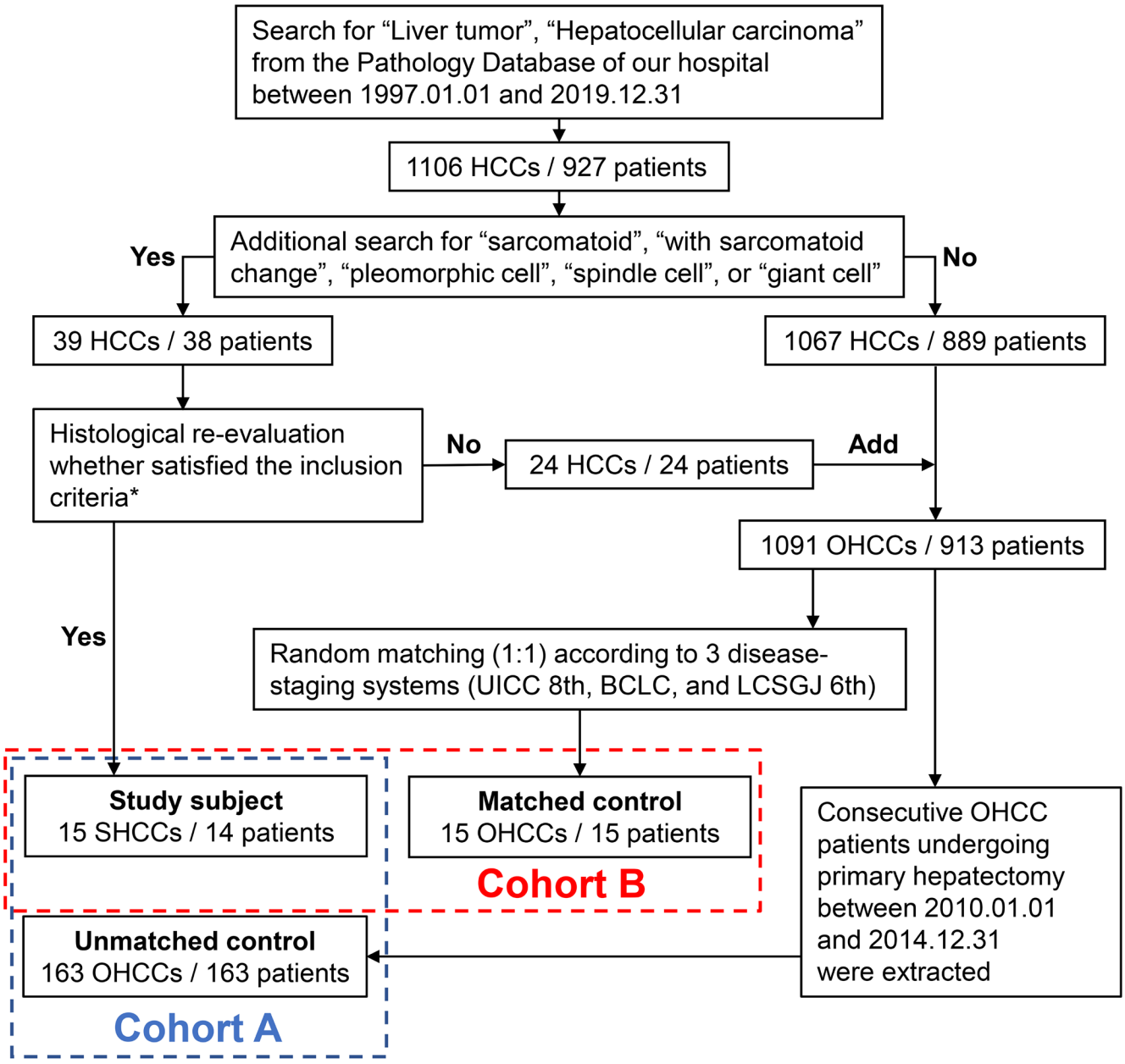

Figure S1. Flow chart of case selection.

The flow chart shows the process of case selection in cohorts A and B. Clinicopathological and prognostic analyses were mainly performed in cohort A, whereas tumor-specific analyses were mainly performed in cohort B. The inclusion criteria (shown by an asterisk in the flow chart) for SHCC was as follows: HCC with at least a 10% sarcomatous component.

Abbreviations: HCC, hepatocellular carcinoma; SHCC, sarcomatoid hepatocellular carcinoma; OHCC, ordinary hepatocellular carcinoma; UICC, Union for International Cancer Control; BCLC, Barcelona Clinic Liver Cancer; LCSGJ, Liver Cancer Study Group of Japan.

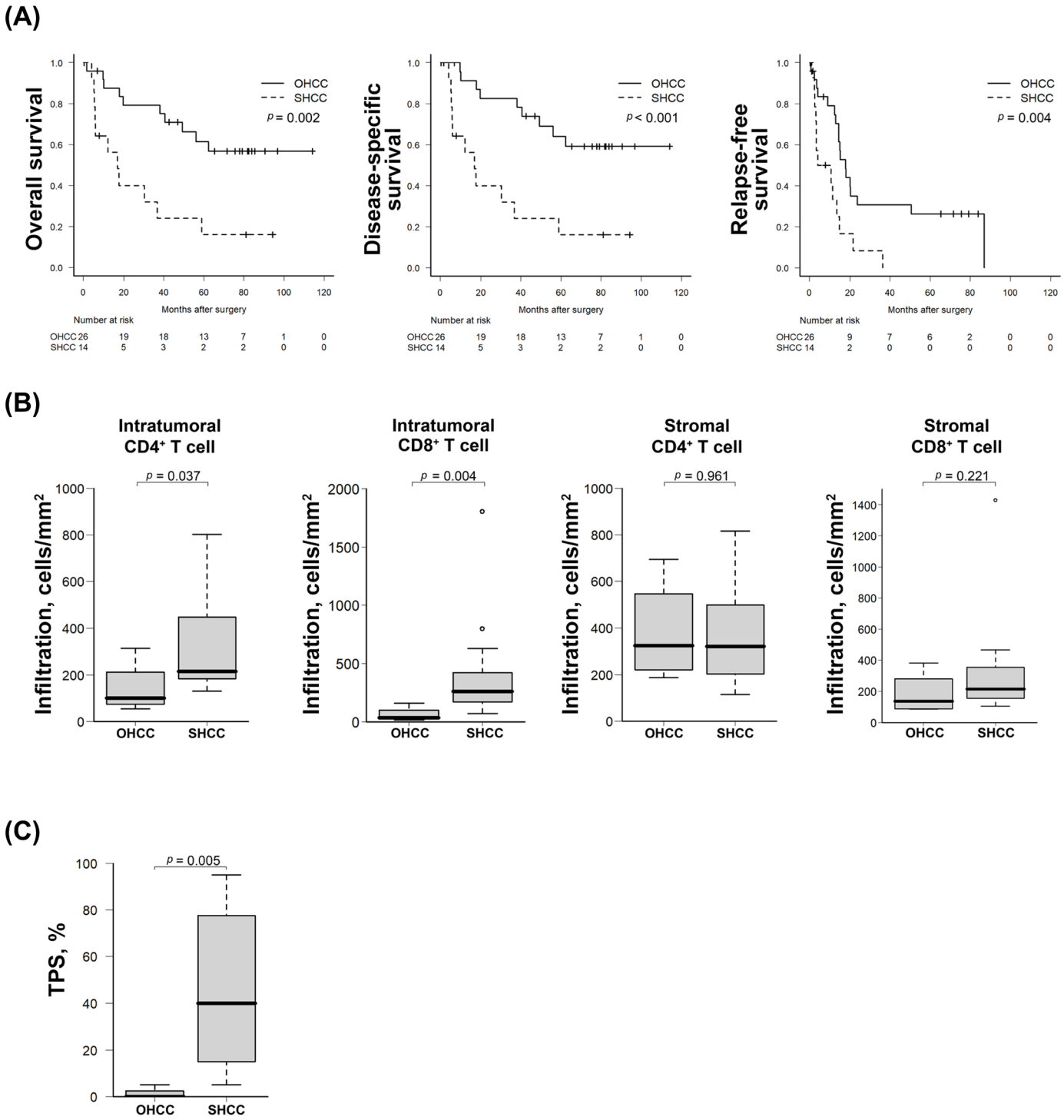

Figure S2. Comparisons of prognostic outcomes and immune profiles between poorly differentiated OHCCs and SHCCs.

(A) Kaplan–Meyer estimates show significantly poorer OS, DSS, and RFS between 14 patients with SHCC and 26 patients with poorly differentiated OHCC in cohort B ($p = 0.002$, $p < 0.001$, and $p = 0.004$, respectively).

(B) Boxplots show the significantly higher density of intratumoral $CD4^+$ and $CD8^+$ T cells in SHCCs (n = 15) than poorly differentiated OHCCs (n = 4) ($p = 0.037$ and $p = 0.004$, respectively). The difference in the densities of stromal T cells was not statistically significant.

(C) Boxplot shows the significantly higher TPS in SHCCs (n = 15) than in OHCCs (n = 4) (p = 0.005).

Abbreviations: SHCC, sarcomatoid hepatocellular carcinoma; OHCC, ordinary hepatocellular carcinoma; OS, overall survival; DSS, disease-specific survival; RFS, relapse-free survival; TPS, tumor proportion score.

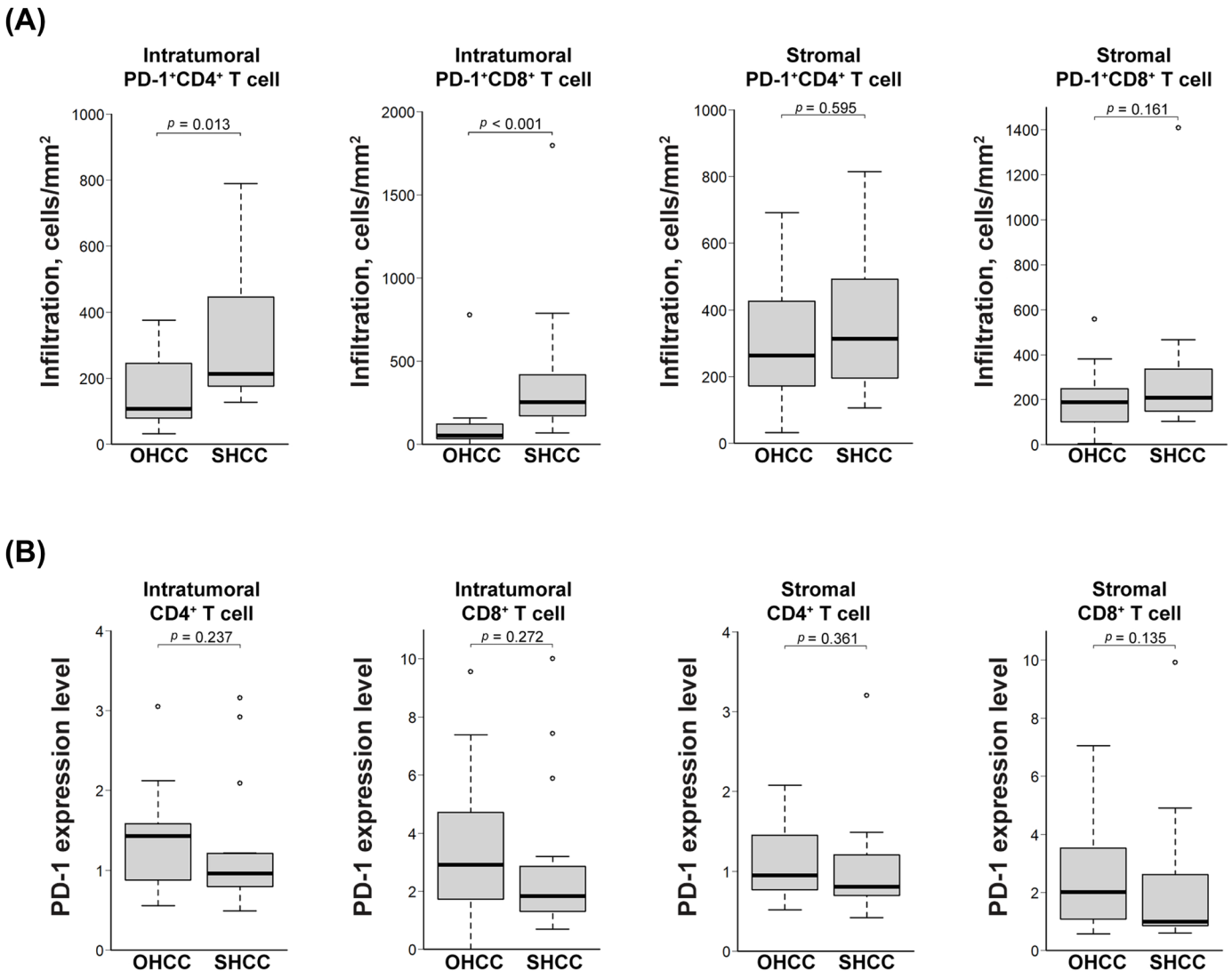

Figure S3. Comparisons of the density of intratumoral and stromal PD-1$^+$ T cells and the level of PD-1 expression on T cells between SHCCs and OHCCs.

(A) Boxplots show that the density of intratumoral PD-1$^+$CD4$^+$ and PD-1$^+$CD8$^+$ cells in SHCCs was significantly higher than that in OHCCs (p = 0.013 and p < 0.001, respectively), whereas the density of stromal PD-1$^+$CD4$^+$ and PD-1$^+$CD8$^+$ T cells was not significantly different in SHCCs and OHCCs.

(B) PD-1 expression level on intratumoral and stromal CD4$^+$/CD8$^+$ T cells was not significantly different between SHCCs and OHCCs.

Abbreviations: OHCC, ordinary hepatocellular carcinoma; SHCC, sarcomatoid hepatocellular carcinoma; PD-1, programmed death-1.

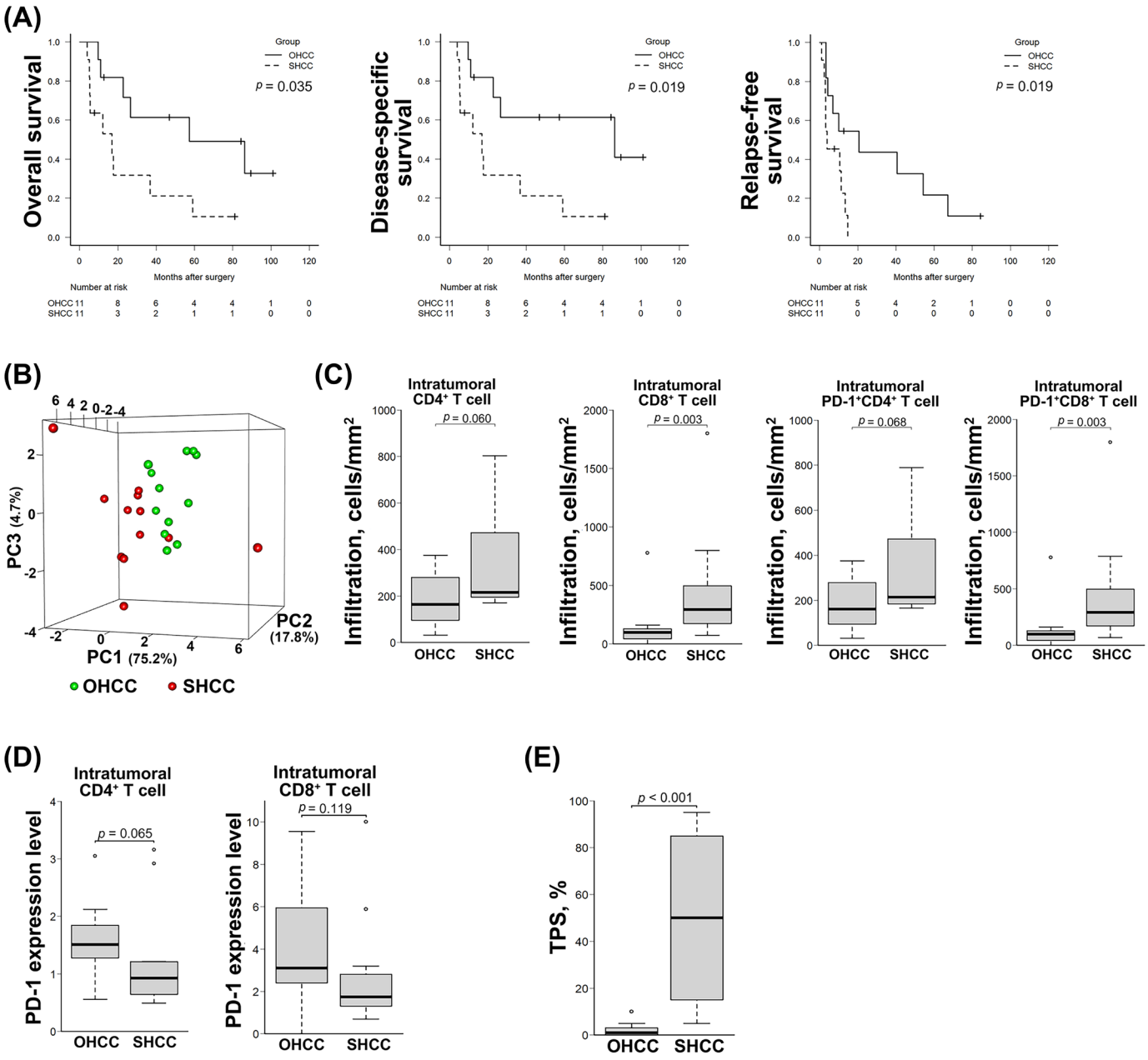

Figure S4. Comprehensive analyses of prognostic outcomes and immune profiles in treatment-naive cases.

(A) Even in the treatment-naive cohort, SHCC patients showed significantly poorer prognostic outcomes regarding OS (p = 0.035), DSS (p = 0.019), and RFS (p = 0.019) than OHCC patients.

(B) The principal component analysis based on the quantitative immune profile of treatment-naive cases shows the discrimination between SHCCs and OHCCs. The proportion of variance in the first, second, and third principal component was 75.2%, 17.8%, and 4.7%, respectively. Green and red spheres indicate OHCCs and SHCCs, respectively.

(C) The density of intratumoral $CD8^+$ T cells and $PD\text{-}1^+CD8^+$ T cells was significantly higher in SHCCs than OHCCs (p = 0.003 and p = 0.003, respectively), whereas the difference in the density

of intratumoral $CD4^+$ and $PD\text{-}1^+CD4^+$ T cells was not statistically significant between the two groups ($p = 0.060$ and $p = 0.068$, respectively).

(D) PD-1 expression level on intratumoral $CD4^+$ and $CD8^+$ T cells was not significantly different between SHCCs and OHCCs ($p = 0.065$ and $p = 0.119$, respectively).

(E) The boxplot shows that the TPS of SHCCs was significantly higher than that of OHCCs ($p < 0.001$).

Abbreviations: OS, overall survival; DSS, disease-specific survival; RFS, relapse-free survival; OHCC, ordinary hepatocellular carcinoma; SHCC, sarcomatoid hepatocellular carcinoma; PD-1, programmed death-1; PC, principal component; TPS, tumor proportion score.