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High serum soluble CD155 level predicts poor prognosis and correlates with an immunosuppressive tumor microenvironment in hepatocellular carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies with poor prognosis. There is no research about the clinical significance of serum soluble CD155 (sCD155) level for HCC. We aim to explore the prognostic and diagnostic value of sCD155 in HCC patients undergoing curative resection.

Methods: Serum sCD155 level in HCC patients was determined by enzyme-linked immunosorbent assay. The prognostic significance of sCD155 was evaluated by Cox regression and Kaplan–Meier analyses. CD155 expression and biomarkers of immune cells in HCC tissues were detected by immunohistochemistry staining. The diagnostic significance of sCD155 was evaluated using receiver operating characteristic curve.

Results: Serum sCD155 level was significantly increased in HCC patients and predicted poor prognosis. The prognostic value of sCD155 remained in low recurrent risk subgroups of HCC. Serum sCD155 level was positively related to CD155 expression in HCC tissues. High serum sCD155 level was associated with decreased numbers of CD8^{+}T cells and CD56^{+}NK cells and increased number of CD163^{+}M2 macrophages. Serum sCD155 level had better performance in distinguishing HCC patients from healthy donors and patients with chronic liver conditions than α-fetoprotein. Among patients with α-fetoprotein ≤ 20 ng/ml, serum sCD155 level could differentiate HCC patients from non-HCC patients.

An-Li Jin, Yi-Hui Yang and Xi Su contributed equally to this study.

Conclusion: Serum sCD155 level represents a promising biomarker for diagnosis and prognosis of HCC. High serum sCD155 level may reflect an immunosuppressive tumor microenvironment in HCC.

KEYWORDS

biomarker, diagnosis, hepatocellular carcinoma, immunosuppression, prognosis, soluble poliovirus receptor

1 | BACKGROUND

Hepatocellular carcinoma (HCC) is a malignant solid tumor, and the incidence and prevalence rates of HCC are increasing.[1] Curative resection is the main treatment strategy for HCC; however, the 5-year survival rate of HCC patients after surgery remains low because of the high rates of recurrence and metastasis.[2] Additionally, many HCC patients are diagnosed at advanced stages due to the lack of specific biomarkers for early-stage HCC and thus are not candidates for surgery, resulting in a 5-year survival rate of <16%.[3,4] Therefore, identifying novel biomarkers for prognosis prediction and early diagnosis of HCC is critical.

Poliovirus receptor (PVR/CD155) is initially identified as a receptor for the poliovirus.[5] CD155 is expressed at low levels in most normal tissues, but it is frequently overexpressed in tumor tissues of various cancers, including colorectal cancer,[6] breast cancer,[7] etc. Recent studies have reported the cell-intrinsic role of CD155 in promoting tumor progression through modulating cell adhesion, migration and polarization,[8] and the cell-extrinsic role of CD155 in immune regulation.[9] CD155 is the ligand for both co-stimulatory receptor (DNAM-1) and co-inhibitory receptors (TIGIT and CD96) on T cells and natural killer (NK) cells,[10] and the balance between co-stimulatory and co-inhibitory signals in tumor microenvironment (TME) is often disturbed, resulting in an immunosuppressive TME.[11] Sun et al[12] found that CD155 was overexpressed and predicted poor prognosis in HCC, and CD155-CD96 interaction resulted in NK cell exhaustion. A previous study showed that anti-CD155 monoclonal antibody reduced lung metastasis in osteosarcoma in vivo, suggesting that CD155 might be a promising target for immunotherapy.[13] Recent studies reported the presence of soluble CD155 (sCD155) in serum, and serum CD155 level was significantly higher in cancer patients than levels in healthy donors.[14] These studies indicated that sCD155 could be a promising biomarker for diagnosis and prognosis of various cancers. However, the clinical significance of sCD155 for HCC patients has not been investigated.

In this study, we conducted a retrospective study to explore the potential clinical significance of sCD155 in HCC patients undergoing curative resection and investigated the correlation between serum sCD155 level and immune cell infiltration in TME. Our results indicated that serum sCD155 level was expected to be a novel biomarker for diagnosis and prognosis of HCC, which might provide valuable application for the management of HCC patients.

2 | METHODS AND MATERIALS

2.1 | Clinical specimens and follow-up

Blood samples from 355 participants were collected from 2018 to 2019 in the Department of Laboratory Medicine, Zhongshan Hospital, Fudan University. All specimens were defined as five groups: hepatocellular carcinoma (HCC, $n = 148$, HCC cohort 1), cirrhosis (CIS, $n = 51$), chronic hepatitis B (CHB, $n = 59$), intrahepatic cholangiocarcinoma (ICC, $n = 44$), and healthy donors (HD, $n = 53$). For the analysis of immune cell infiltration, tumor tissues and corresponding blood samples from 24 HCC patients undergoing curative resection (HCC cohort 2) from 2019 to 2020 in the Liver Cancer Institute, Zhongshan Hospital, Fudan University, were collected. All HCC patients enrolled in this study were diagnosed by hematoxylin-eosin staining and had not accepted prior anti-cancer treatment. China liver cancer (CNLC) staging system was used to determine tumor stage.[15] Complete clinical information of each patient had been obtained. Survival data were collected until June 2021. Time to recurrence (TTR) was defined as the time between the liver curative resection and the first time of discovering intrahepatic recurrence or extrahepatic metastasis. Approval for the use of human specimens was obtained from the Research Ethics Committee of Zhongshan Hospital, Fudan University. Informed consent had been obtained from all patients.

2.2 | Blood samples

Blood samples were collected before the surgery. A total of 3-ml peripheral blood was centrifuged at 4°C, 2000 × **g** for 10 min to eliminate cells, followed by centrifugation at 4°C, 13,000 × **g** for 15 min to eliminate other cellular organelles. Samples were aliquoted and stored at −80°C until analysis. Routine laboratory parameters were measured in the Department of Laboratory Medicine, Zhongshan Hospital, Fudan University. Systemic inflammation index (SII, platelet ×neutrophil/lymphocyte) was defined according to our previous report.[16]

2.3 | Enzyme-linked immunosorbent assay (ELISA)

Serum sCD155 level was detected by ELISA according to manufacturer's instructions (Poliovirus receptor ELISA kit, USCN Life Science, Wuhan, China). Samples were blind to the person who performed experiments. A cut-off value for serum sCD155 level was set at 4218.08 ng/ml, which was the median value of sCD155 concentration in HCC cohort 1 (n = 148).

2.4 | Immunohistochemistry (IHC) staining

Paraffin-embedded HCC tissues were made into sections. IHC staining assay was carried out using standard procedures as previously described.[17] The antibodies used in this study were as follows: CD155 (1:200, Cell Signaling Technology, CST, USA), CD8 (1:200, CST), FOXP3 (1:100, CST), CD56 (1:200, Abcam, USA), and CD163 (1:200, CST). Histochemistry score (H-score) = (percentage of weak intensity area ×1) + (percentage of moderate intensity area ×2) + (percentage of strong intensity area ×3).[18,19] Two pathological investigators who were blind to samples evaluated the numbers of positive immune cells on sections. If there was any discrepancy between their results, it would be resolved by consensus. The average of the numbers of positive immune cells (cells/mm^2) was used as the quantification results of particular cell population.[20]

2.5 | Statistical analysis

SPSS software 16.0 and Graphpad prism 8.0 were used for statistical analysis. Experimental values for continuous variables were expressed as the mean ± standard deviation (SD). The chi-squared test, Fisher's exact test, Student's t test, Mann–Whitney U test, and Wilcoxon signed-rank test were used to evaluate the significance of differences in data between groups. Kaplan–Meier survival curves and log-rank test were used to assess TTR of HCC patients. Univariate and multivariate analyses were performed via establishing Cox proportional hazards models. Receiver operating characteristic (ROC) curve and area under curve (AUC) were constructed to evaluate the diagnostic value of biomarkers in HCC patients. The corresponding sensitivity and specificity were recorded with the largest Youden index. P < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Serum sCD155 level is increased in HCC patients and correlates with advanced tumor stage

Serum sCD155 levels in all five groups (HCC, CIS, CHB, HD, and ICC) were listed in Table 1. Our results showed that serum sCD155 level was higher in HCC patients compared with levels in other groups, and serum sCD155 level increased with disease progression (Figure 1A).

TABLE 1 The levels of sCD155 and AFP in the serum of participants (n = 355) in our study

Groups	N	AFP, ng/ml (mean ± SD)	sCD155, pg/ml (mean ± SD)
HCC	148	3375.9 ± 11367.0	4670.5 ± 2264.4
CIS	51	54.3 ± 223.0	2460.3 ± 1055.8
CHB	59	15.5 ± 72.2	1972.0 ± 940.7
HD	53	3.6 ± 1.9	1781.5 ± 820.6
ICC	44	80.0 ± 236.5	2802.5 ± 1462.8

Abbreviations: AFP, α-fetoprotein; CHB, chronic hepatitis B; CIS, cirrhosis; HCC, hepatocellular carcinoma; HD, healthy donor; ICC, intrahepatic cholangiocarcinoma; sCD155, soluble CD155; SD, standard deviation.

We also found that serum sCD155 level in the ICC group was higher than the level in the HD group, but it was lower than that of HCC patients (Figure 1A).

The median value of serum sCD155 level was set as cut-off value (4218.08 ng/ml), and HCC patients were divided into high sCD155 level group (sCD155^{high} = 74) and low sCD155 level group (sCD155^{low} = 74). The serum sCD155 level in early-stage HCC (CNLC I-II) patients was lower than that of late-stage HCC (CNLC III) patients (Figure 1B). Consistent with these findings, the percentage of sCD155^{high} patients was higher in the late-stage HCC group than that in the early-stage HCC group (Figure 1C).

3.2 | High serum sCD155 level predicts poor prognosis in HCC patients

We next analyzed the correlation between serum sCD155 level and the clinical characteristics of HCC patients. High serum sCD155 level was associated with incomplete tumor encapsulation, vascular invasion, and advanced CNLC stage (Figure 2A-C; Table 2). We also found higher serum sCD155 levels in HCC patients with recurrence compared with levels in patients without recurrence (Figure 2D).

We then investigated the prognostic significance of serum sCD155 level in HCC patients. Kaplan–Meier analysis showed that sCD155^{high} patients had a shorter TTR compared with sCD-155^{low} patients (Figure 2E). We also explored the prognostic value of sCD155 in the low recurrent risk subgroups of HCC.[21] The prognostic significance of sCD155 remained in the α-fetoprotein (AFP) ≤20 ng/ml subgroup and single tumor lesion subgroup (Figure 2F-G).

Univariate analysis showed that AFP, γ-glutamyl transpeptidase (γ-GT), aspartate aminotransferase (AST), platelet-lymphocyte ratio (PLR), neutrophil-lymphocyte ratio (NLR), SII, tumor size, tumor number, satellite lesions, CNLC stage, and sCD155 level (HR = 1.962, 95%CI = 1.158–3.324, p = 0.012) were related to TTR of HCC patients (Figure 3A; Table 3). Multivariate analysis demonstrated that high serum sCD155 level (HR = 2.212, 95%CI = 1.268–3.857, p = 0.005) was an independent prognostic indicator for predicting TTR in HCC patients (Figure 3B; Table 3).
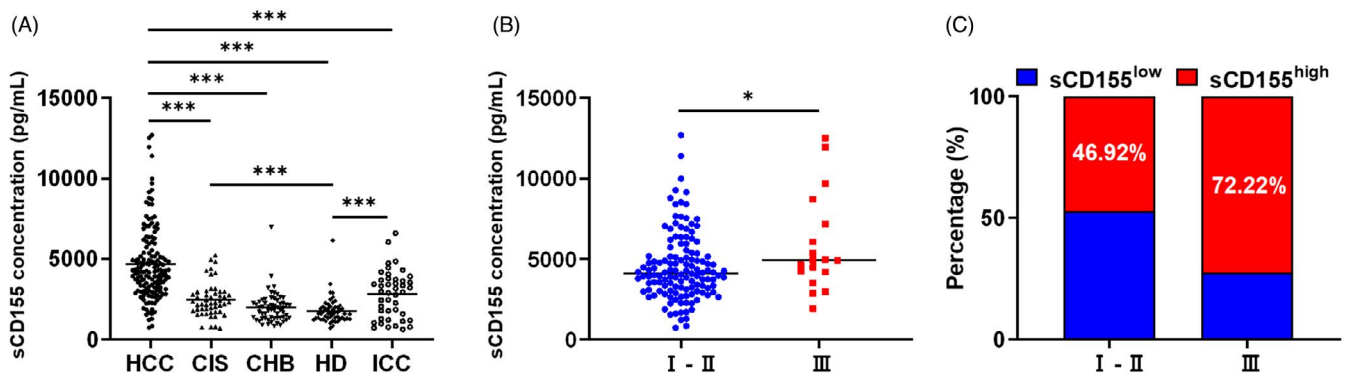
FIGURE 1 Serum sCD155 level is elevated and associated with advanced tumor stage in HCC. A, Serum sCD155 level in groups of HCC, CIS, CHB, HD, and ICC. B, Serum sCD155 level in patients with different CNLC stages. C, Percentages of sCD155^{low} or sCD155^{high} HCC patients in different CNLC stages. *p value < 0.05, **p value < 0.01, and ***p value < 0.001

3.3 | High serum sCD155 level is associated with decreased numbers of CD8^{+}T cells and CD56^{+}NK cells and increased number of CD163^{+}M2 macrophages in HCC tissues

According to the cut-off value of serum sCD155 level, we next classified 24 HCC patients (tumor tissues and corresponding blood samples) into sCD155^{high} (n = 12) group and sCD155^{low} (n = 12) group. We found that CD155 expression in HCC tissues was higher in sCD155^{high} patients compared with sCD155^{low} patients (Figure 4A). We further analyzed the correlation between serum sCD155 level and CD155 expression in HCC tissues, and the results showed that serum sCD155 level was positively related to CD155 expression in HCC tissues (Figure 4B).

A previous study reported a significant role of CD155 in immunosuppressive TME.[11] We therefore next explored the relationship between serum sCD155 level and immune microenvironment status in HCC tissues. Representative images of IHC staining for CD8^{+}, CD56^{+}, FOXP3^{+}, and CD163^{+} cells in HCC tissues were shown in Figure 4C. The results demonstrated that the numbers of CD8^{+}T cells and CD56^{+}NK cells (anti-tumor lymphocytes) in HCC tissues were decreased in sCD155^{high} patients compared with those in sCD155^{low} patients, while the number of CD163^{+}M2 macrophages (immunosuppressive cells) was increased (Figure 4D).

3.4 | Diagnostic value of serum sCD155 level in HCC patients

We next explored the diagnostic value of sCD155 in HCC patients using ROC curve. AFP is a well-established biomarker for the diagnosis of HCC,[22] and thus, we first analyzed the association between the levels of sCD155 and AFP in the serum of HCC patients. We found no significant correlation between the serum levels of AFP and sCD155 in HCC patients (Figure 5A), suggesting that serum sCD155 level might be an ideal biomarker for the diagnosis of HCC

in addition to serum AFP level. Based on ROC curve, we found that sCD155 showed higher accuracy (AUC = 0.8949 [0.8572–0.9326]) than AFP (AUC = 0.8218 [0.7731–0.8705]) in distinguishing HCC patients from non-HCC patients; the AUC values of sCD155 for distinguishing HCC from HD, CHB, and CIS were 0.9301 (0.8894–0.9709), 0.9132 (0.8699–0.9565), and 0.8370 (0.7753–0.8988), respectively (Figure 5B-E; Table 4). These findings indicated that serum sCD155 level was superior to AFP for the diagnosis of HCC. In addition, the combination of sCD155 and AFP achieved better diagnostic performance than any single marker (Figure 5B-E; Table 4). More importantly, among patients with AFP ≤ 20 ng/ml, serum sCD155 level could also differentiate HCC patients from non-HCC patients; the AUC value was 0.8857 (0.8297–0.9417) (Figure 5F; Table 4).

4 | DISCUSSION

HCC is one of the most leading causes of cancer-related death worldwide.[1] In the patients diagnosed with HCC at an early stage, the 5-year survival rate is >70%.[23] However, most HCC patients are diagnosed at a late stage and the 5-year survival of these patients is poor.[3] Previous studies reported the increased level of sCD155 in the serum of cancer patients, including in lung cancer, gastric cancer, colorectal cancer, etc.[14] However, serum sCD155 level has not been previously investigated in HCC.

CD155 functions as a multi-functional molecule in human cancers.[24] Previous studies reported that CD155 promoted cell proliferation through Ras-Raf-MEK-ERK signaling pathway[25] and contributed to mesenchymal phenotype in breast cancer.[26] In addition, CD155 overexpression could induce tumor immune escape via interacting with co-inhibitory receptors on T cells and NK cells.[12,27] All of these findings implied that the effect of CD155 in TME was immunosuppressive in human cancers. However, the assessment of CD155 in tumor tissues is not possible without surgery. In contrast, the evaluation of serum sCD155 level is a low-cost and easy method
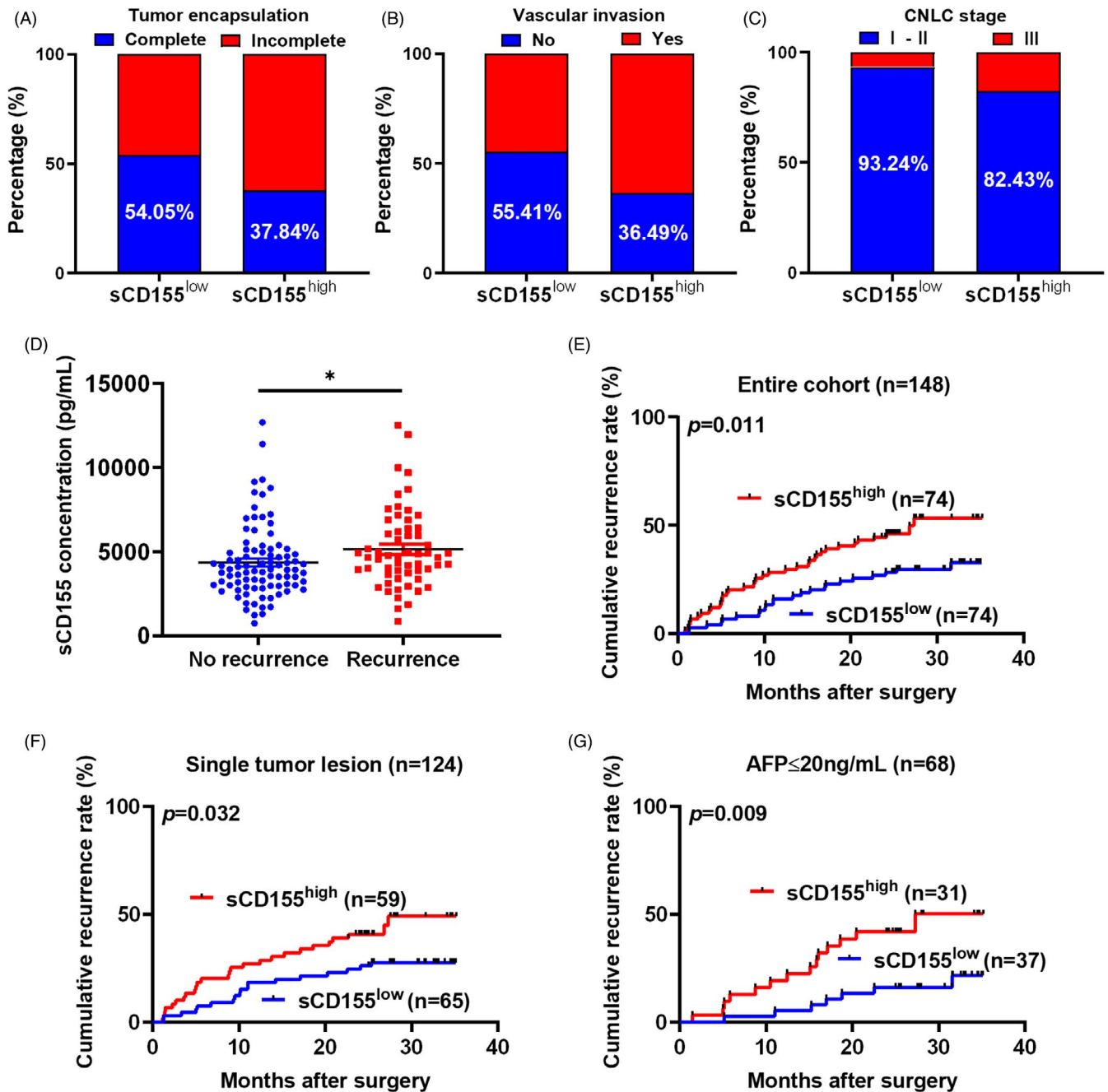
FIGURE 2 Serum sCD155 level is prognostically significant. A-C, Correlation between serum sCD155 level and clinical characteristics of HCC patients (n = 148). D, Serum sCD155 level in HCC patients with or without recurrence. E-G, Kaplan–Meier analysis of TTR of HCC patients in entire HCC cohort, single tumor lesion subgroup and AFP ≤ 20 ng/ml subgroup according to serum sCD155 level. *p value < 0.05, **p value < 0.01, and ***p value < 0.001

for diagnosis and prognosis of HCC. To the best of our knowledge, we first provide evidence for the diagnostic and prognostic value of serum sCD155 level in HCC.

In this study, we found that serum sCD155 level was higher in HCC patients and correlated with advanced tumor stage; these results were in accordance with a previous report in gastric cancer.[14] Our results also showed that high serum sCD155 level was related to advanced HCC stage (CNLC III), indicating that high serum sCD155 level was associated with the aggressive phenotype of HCC;

these findings were in accordance with a previous study in breast cancer.[28] Importantly, we found that sCD155high patients had a shorter TTR than sCD155low patients in HCC. HCC is a highly heterogeneous disease,[29] and thus, we also examined the prognostic significance of sCD155 in low recurrent risk subgroups of HCC. AFP is a well-established marker for monitoring recurrence and metastasis of HCC patients.[30] However, there has been a lack of ideal biomarkers for HCC patients with normal AFP levels.[31] We found that the prognostic value of sCD155 remained in the AFP ≤ 20 ng/ml subgroup,

TABLE 2 Correlation between serum sCD155 level and clinical characteristics of HCC patients (n = 148)

Clinical characteristics	N	sCD155 level Low (n = 74)	sCD155 level High (n = 74)	p
Sex				
Male	119	60	59	0.836
Female	29	14	15	
Age, years				
≤50	49	29	20	0.116
>50	99	45	54	
HBsAg				
Negative	29	15	14	0.836
Positive	119	59	60	
AFP, ng/ml				
≤20	68	37	31	0.322
>20	80	37	43	
PIVKA-II, mAU/ml				
≤40	29	17	12	0.300
>40	119	57	62	
γ-GT, U/L				
≤45	74	41	33	0.188
>45	74	33	41	
AST, U/L				
≤35	100	46	54	0.160
>35	48	28	20	
CEA, ng/ml				
≤5	135	69	66	0.384
>5	13	5	8	
CA199, U/ml				
≤34	127	63	64	0.814
>34	21	11	10	
Platelet, 10^9/L				
≤300	137	67	70	0.347
>300	11	7	4	
PLR				
≤150	113	55	58	0.562
>150	35	19	16	
NLR				
≤5	126	65	61	0.355
>5	22	9	13	
SII				
≤330	72	36	36	1.000
>330	76	38	38	
Liver cirrhosis				
No	72	37	35	0.742
Yes	76	37	39	
Tumor size, cm				
≤5	84	43	41	0.740
>5	64	31	33	

TABLE 2 (Continued)

Clinical characteristics	N	sCD155 level Low (n = 74)	sCD155 level High (n = 74)	p
Tumor number				
Single	124	65	59	0.181
Multiple	24	9	15	
Satellite lesion				
No	131	67	64	0.439
Yes	17	7	10	
Tumor encapsulation				
Complete	68	40	28	**0.048**
Incomplete	80	34	46	
Vascular invasion				
No	68	41	27	**0.021**
Yes	80	33	47	
Edmondson stage				
I-II	75	42	33	0.139
III-IV	73	32	41	
CNLC stage				
I-II	130	69	61	**0.044**
III	18	5	13	

Abbreviations: AFP, α-fetoprotein; AST, aspartate transaminase; CA199, carbohydrate antigen 199; CEA, carcinoembryonic antigen; CNLC, China liver cancer; HBsAg, hepatitis B surface antigen; NLR, neutrophil-lymphocyte ratio; PIVKA-II, protein induced by vitamin K absence or antagonist-II; PLR, platelet-lymphocyte ratio; PLT, platelet; sCD155, soluble CD155; SII, systemic immune-inflammation index; γ-GT, γ-glutamyl transpeptidase.

The bold values were considered statistically significant (p<0.05).

which contributed to identifying AFP-negative patients with a high risk of recurrence in HCC.

A previous study reported that a higher level of sCD155 was associated with better response to chemotherapy and favorable overall survival in esophageal cancer, which was not consistent with our results.[32] One possible reason for this discrepancy was the difference in tumor types. In addition, the previous study had a small cohort size (n = 47), which was not sufficient to obtain a definitive conclusion.

AFP is the most widely used biomarker for the diagnosis of HCC.[22] However, approximately 40% of early-stage HCC patients and 15%-20% of advanced stage HCC patients are AFP-negative (AFP ≤ 20 ng/ml).[33] In our study, we found that sCD155 showed better performance compared with AFP in distinguishing HCC patients from other groups, and the combination of sCD155 and AFP achieved better diagnostic performance than any single marker. Importantly, the diagnostic significance of serum sCD155 level in HCC patients with AFP ≤ 20 ng/mL that was demonstrated in this study indicated that serum sCD155 level might help clinicians identify HCC patients with normal AFP level that otherwise would escape diagnosis or be misdiagnosed.
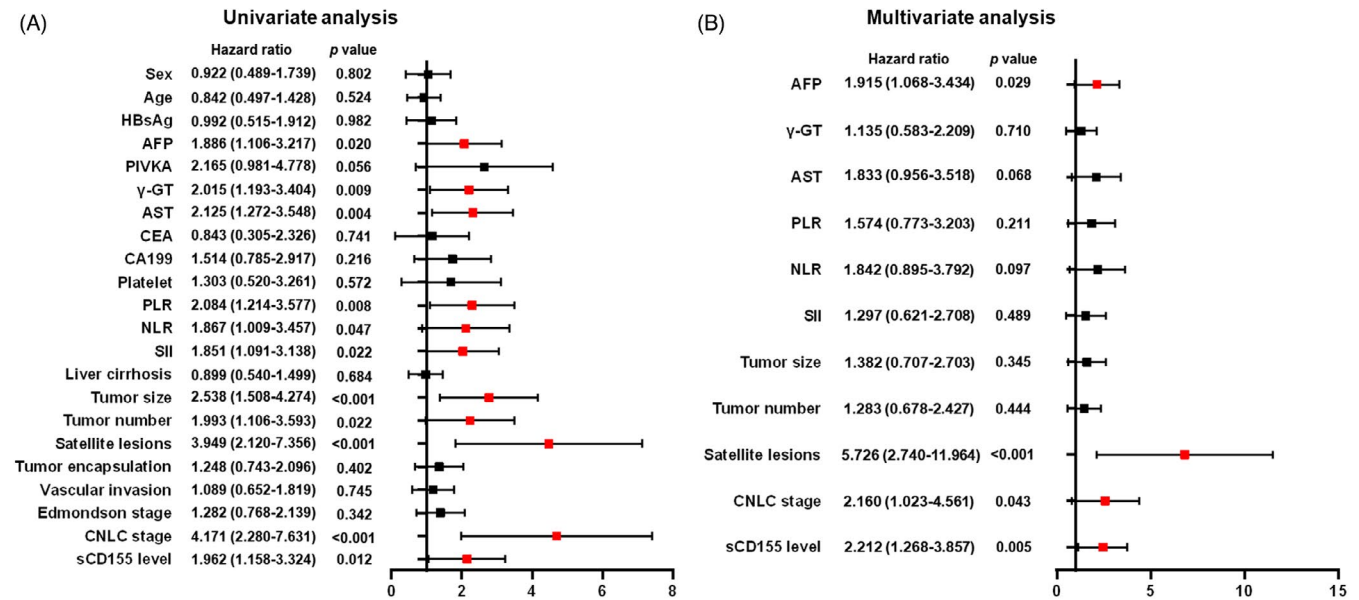
FIGURE 3 Metanalysis plot of univariate and multivariate Cox proportional regression analysis of factors associated with recurrence. A, Univariate analysis. B, Multivariate analysis

TABLE 3 Cox proportional regression analysis of factors associated with recurrence in HCC patients ($n = 148$)

Variables	Recurrence			
	Univariate		Multivariate	
	HR (95% CI)	p	HR (95% CI)	p
Sex (Male vs. Female)	0.922 (0.489–1.739)	0.802	N.A.	N.A.
Age (>50 years vs. ≤50 years)	0.842 (0.497–1.428)	0.524	N.A.	N.A.
HBsAg (Positive vs. Negative)	0.992 (0.515–1.912)	0.982	N.A.	N.A.
AFP (>20ng/ml vs. ≤20 ng/ml)	1.886 (1.106–3.217)	**0.020**	1.915 (1.068–3.434)	**0.029**
PIVKA-II (>40 mAU/ml vs. ≤40 mAU/ml)	2.165 (0.981–4.778)	0.056	N.A.	N.A.
γ-GT (>45 U/L vs. ≤45 U/L)	2.015 (1.193–3.404)	**0.009**	1.135 (0.583–2.209)	0.710
AST (>35 U/L vs. ≤35 U/L)	2.125 (1.272–3.548)	**0.004**	1.833 (0.956–3.518)	0.068
CEA (>5 ng/ml vs. ≤5 ng/ml)	0.843 (0.305–2.326)	0.741	N.A.	N.A.
CA199 (>34 U/ml vs. ≤34 U/ml)	1.514 (0.785–2.917)	0.216	N.A.	N.A.
Platelet (>300 × 10^9/L vs. ≤300 × 10^9/L)	1.303 (0.520–3.261)	0.572	N.A.	N.A.
PLR (>150 vs. ≤150)	2.084 (1.214–3.577)	**0.008**	1.574 (0.773–3.203)	0.211
NLR (>5 vs. ≤5)	1.867 (1.009–3.457)	**0.047**	1.842 (0.895–3.792)	0.097
SII (>330 vs. ≤330)	1.851 (1.091–3.138)	**0.022**	1.297 (0.621–2.708)	0.489
Liver cirrhosis (Yes vs. No)	0.899 (0.540–1.499)	0.684	N.A.	N.A.
Tumor size (>5 cm vs. ≤5 cm)	2.538 (1.508–4.274)	**<0.001**	1.382 (0.707–2.703)	0.345
Tumor number (Multiple vs. Single)	1.993 (1.106–3.593)	**0.022**	1.283 (0.678–2.427)	0.444
Satellite lesions (Yes vs. No)	3.949 (2.120–7.356)	**<0.001**	5.726 (2.740–11.964)	**<0.001**
Tumor encapsulation (Incomplete vs. Complete)	1.248 (0.743–2.096)	0.402	N.A.	N.A.
Vascular invasion (Yes vs. No)	1.089 (0.652–1.819)	0.745	N.A.	N.A.
Edmondson stage (III-IV vs. I-II)	1.282 (0.768–2.139)	0.342	N.A.	N.A.
CNLC stage (III vs. I-II)	4.171 (2.280–7.631)	**<0.001**	2.160 (1.023–4.561)	**0.043**
sCD155 level (High vs. Low)	1.962 (1.158–3.324)	**0.012**	2.212 (1.268–3.857)	**0.005**

Abbreviations: 95% CI, 95% confidence interval; AFP, α-fetoprotein; AST, aspartate transaminase; CA199, carbohydrate antigen 199; CEA, carcinoembryonic antigen; CNLC, China liver cancer; HBsAg, hepatitis B surface antigen; HR, hazard ratio; N.A., not applicable; NLR, neutrophil-lymphocyte ratio; PIVKA-II, protein induced by vitamin K absence or antagonist-II; PLR, platelet-lymphocyte ratio; PLT, platelet; sCD155, soluble CD155; SII, systemic immune-inflammation index; γ-GT, γ-glutamyl transpeptidase.

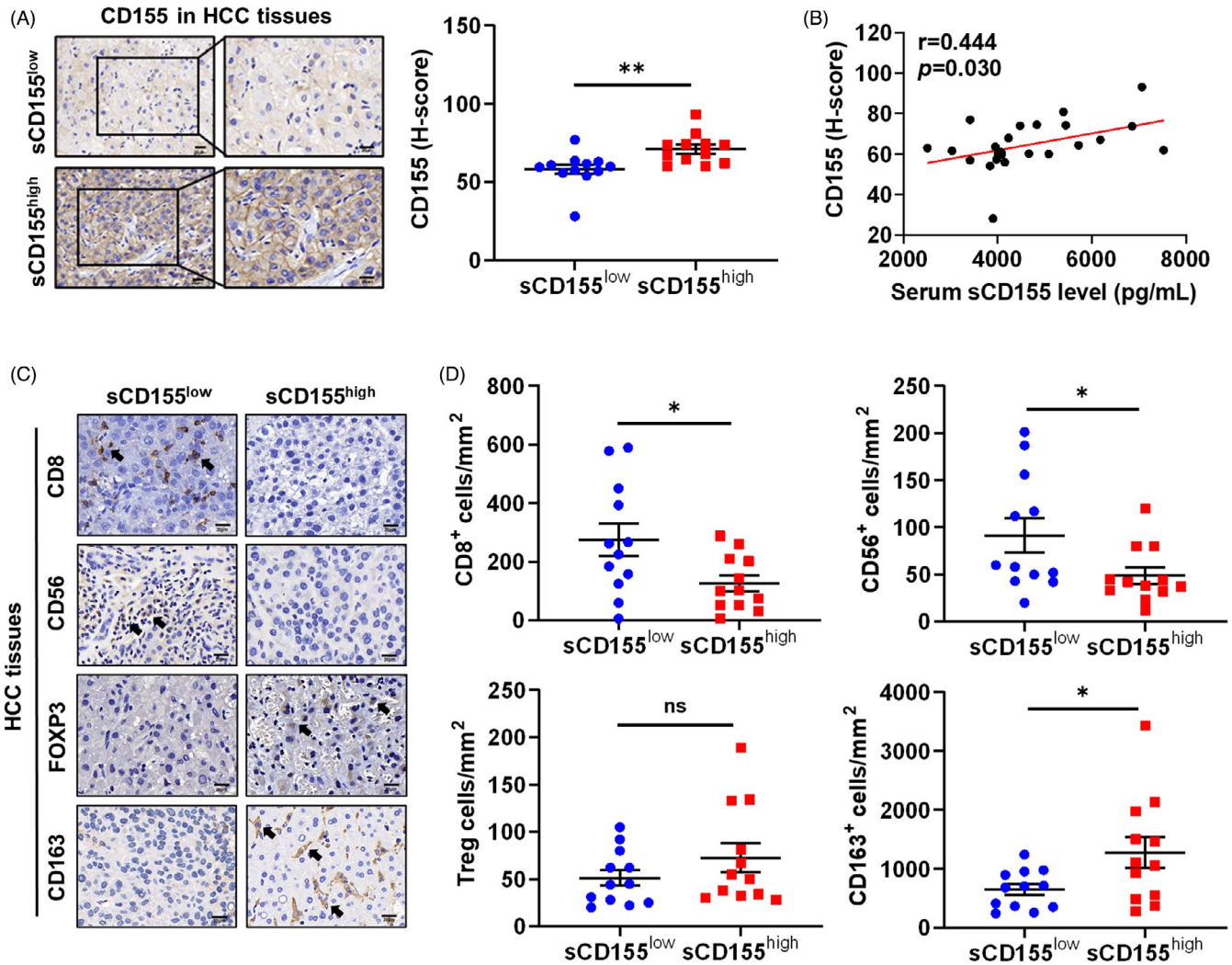The bold values were considered statistically significant ($p<0.05$).

FIGURE 4 High serum sCD155 level correlates with an immunosuppressive TME in HCC. A, CD155 expression in HCC tissues was detected by IHC staining. Scale bar: 20 μm. B, Correlation between CD155 expression in HCC tissues and serum sCD155 level in corresponding blood samples. C, Representative IHC staining images of CD8^{+}, CD56^{+}, FOXP3^{+}, and CD163^{+} cells in HCC tissues. Scale bar: 20 μm. D, Numbers of CD8^{+}, CD56^{+}, FOXP3^{+} and CD163^{+} cells in HCC tissues of sCD155^{high} patients and sCD155^{low} patients, respectively. *p value < 0.05, **p value < 0.01, and ***p value < 0.001

TME is an immunosuppressive microenvironment that is composed of innate and adaptive immune cells, stromal cells, endothelial cells, cancer-associated fibroblasts, and cancer cells.[34] CD8^{+}T cells and NK cells are two major effector anti-tumor lymphocyte populations in TME. It had been reported that the numbers of CD8^{+}T cells and CD56^{+}NK cells were decreased in tumor-infiltrating lymphocytes in HCC, and CD8^{+}T cells showed impaired ability for the production of interferon-γ and perforin.[35] Previous studies demonstrated that CD155 reduced anti-tumor immune responses through interacting with CD96 or TIGIT on T cells and NK cells.[12,36] M2 macrophages produced angiogenic molecules, such as vascular endothelial growth factor, platelet-derived growth factor, and transforming growth factor β, leading to the promotion of tumor progression.[37] A previous study reported that M2 macrophages re-forced the expression of oncogenes and stemness, such as CD155 and SRC.[38] In our study, we found that serum sCD155 level was positively related

to CD155 expression in HCC tissues, suggesting that sCD155 in the serum might be derived from primary tumor tissues. Moreover, high serum sCD155 level correlated with decreased numbers of CD8^{+}T cells and CD56^{+}NK cells and increased number of CD163^{+}M2 macrophages, representing an immunosuppressive TME in HCC. Our results suggested that the detection of serum CD155 level might be a low-cost, easy, fast, and non-invasive approach to gain insights into the immune microenvironment status of HCC patients.

This study has several limitations. Our study had a relatively small number of patients, and data from only a single center were used. Therefore, multi-center studies with more HCC patients are needed. Additionally, it should be noted that most HCC patients in China have a hepatitis B virus-positive background, and therefore, the diagnostic and prognostic significance of sCD155 in HCC should be validated in populations from other regions. A recent study reported that sCD155 inhibited DNAM-1-mediated cytotoxic activity
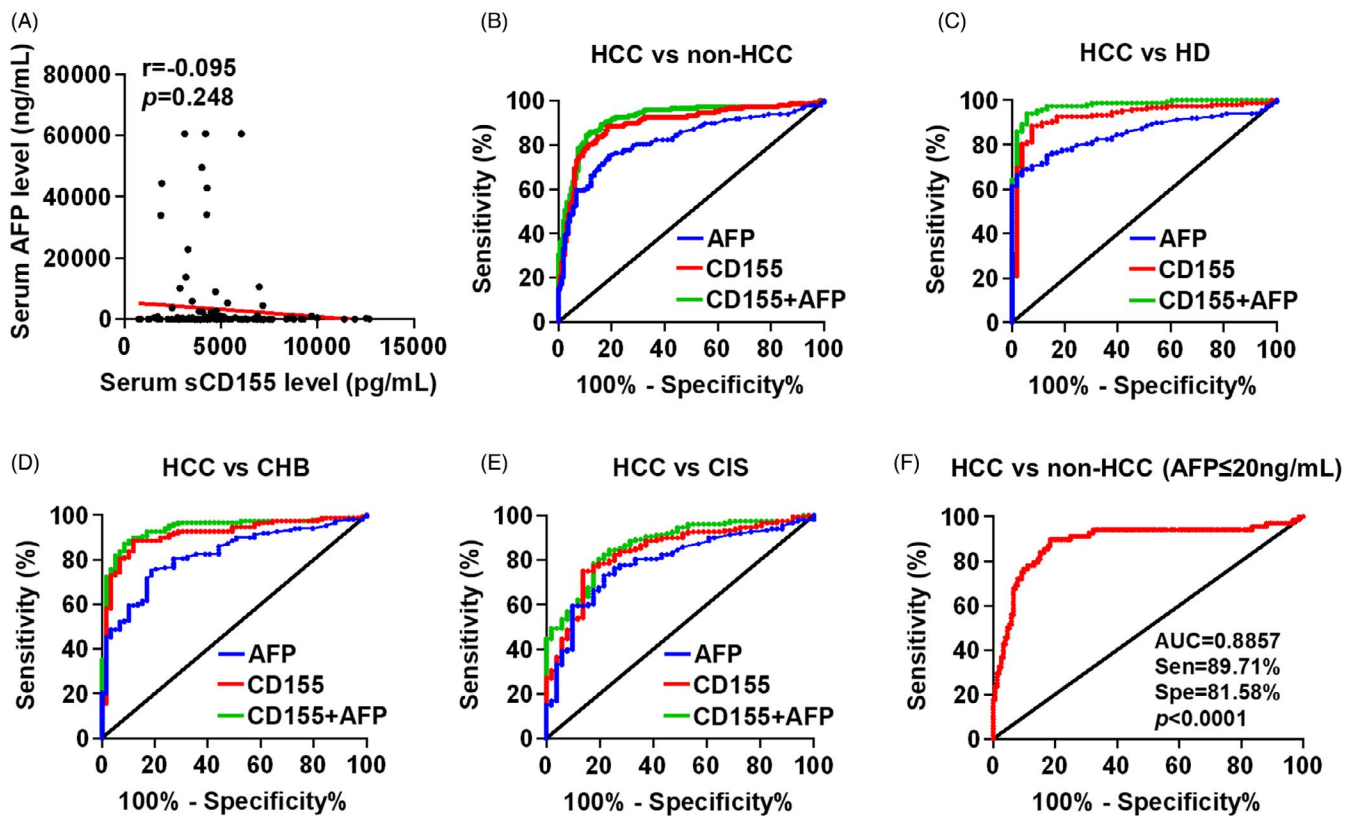
FIGURE 5 The diagnostic value of serum sCD155 level in HCC. A, Correlation between serum sCD155 level and serum AFP level in HCC patients. B-E, The diagnostic performance of sCD155 in distinguishing HCC patients from non-HCC, HD, CHB, and CIS, respectively. F, Among patients with AFP ≤ 20 ng/ml, the diagnostic performance of sCD155 in distinguishing HCC patients from non-HCC patients

TABLE 4 The diagnostic value of serum sCD155 level in HCC

Groups	AUC (95% CI)	Sensitivity (%)	Specificity (%)	p
HCC vs. HD				
AFP	0.8536 (0.8030–0.9042)	68.24	96.23	<0.0001
sCD155	0.9301 (0.8894–0.9709)	88.51	92.45	<0.0001
AFP+sCD155	0.9762 (0.9569–0.9954)	93.92	94.34	<0.0001
HCC vs. CHB				
AFP	0.8229 (0.7646–0.8812)	75.00	81.36	<0.0001
sCD155	0.9132 (0.8699–0.9565)	88.51	88.14	<0.0001
AFP+sCD155	0.9394 (0.9044–0.9744)	87.16	91.53	<0.0001
HCC vs. CIS				
AFP	0.7876 (0.7185–0.8566)	72.97	78.43	<0.0001
sCD155	0.8370 (0.7753–0.8988)	75.00	86.27	<0.0001
AFP+sCD155	0.8678 (0.8147–0.9209)	84.46	76.47	<0.0001
HCC vs. non-HCC				
AFP	0.8218 (0.7731–0.8705)	75.00	80.98	<0.0001
sCD155	0.8949 (0.8572–0.9326)	88.51	81.60	<0.0001
AFP+sCD155	0.9202 (0.8878–0.9526)	85.81	88.34	<0.0001
HCC vs. non-HCC (AFP ≤ 20 ng/ml)				
sCD155	0.8857 (0.8297–0.9417)	89.71	81.58	<0.0001

Abbreviations: 95% CI, 95% confidence interval; AFP, α-fetoprotein; AUC, area under curve; CHB, chronic hepatitis B; CIS, cirrhosis; HCC, hepatocellular carcinoma; HD, healthy donor; sCD155, soluble CD155.

of NK cells, promoting the lung colonization of B16/BL6 melanoma cells.[39] Our data also demonstrated that high serum sCD155 level was related to an immunosuppressive TME in HCC. However, the role and underlying mechanism of sCD155 in immune regulation in HCC require further investigations.

5 | CONCLUSION

Here, we demonstrate the diagnostic and prognostic significance of serum sCD155 level for HCC patients. Our findings suggest that serum sCD155 level is a promising biomarker for the management of HCC patients. High serum sCD155 level is related to an immunosuppressive TME in HCC. Further research into the correlation between sCD155 and immune cell infiltration may provide novel insights into HCC progression.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS
Wei Guo, Xin-Rong Yang, Jia Fan, Jian Zhou, Bai-Shen Pan, and Bei-Li Wang were responsible for study conception and design; An-Li Jin, Wen-Jing Yang, Wei Chen, Tong Li, Lin Ding, and Hao Wang were responsible for material preparation and data collection; An-Li Jin, Yi-Hui Yang, and Xi Su were responsible for data analysis; An-Li Jin, Yi-Hui Yang, Xi Su, Wen-Jing Yang, Te Liu, Wei Guo, and Xin-Rong Yang were responsible for drafting and revision of the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL
The study was approved by the Research Ethics Committee of Zhongshan Hospital, Fudan University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

CONSENT TO PARTICIPATE
Informed consent was obtained from all individual participants included in the study.

DATA AVAILABILITY STATEMENT
All data generated or analyzed during this study are included in this published article [and its supplementary information files (Table S1)].

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SUPPORTING INFORMATION

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